Diversity and community structure of gastrointestinal helminths of *Rhabdomys* spp. and other small mammals in South Africa

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To Arthur and Cailin.

Declaration

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Some of the contents contained in this thesis are taken directly from manuscripts (Chapters 2-5) submitted or drafted for publication in the primary scientific literature. This resulted in some overlap in content between the chapters.

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Abstract

Descriptive information forms the basis for broader ecological questions and therefore plays a vital role in studies that explore patterns in parasite diversity, distribution and species assemblages. Parasite-locality information can further aid in the development of species distribution maps that can be of value in the identification of disease risk and aid proactive disease management. As yet, current knowledge of helminth-host and helminth-locality associations in small mammals in South and southern Africa are scant. As a result, it is uncertain how host and environmental factors shape helminth infections and community structure across a climatically diverse landscape. To address this paucity of information the study aims to: (1) record descriptive information on helminth-host associations and the spatial and temporal distribution of helminth parasites associated with small mammals, (2) using two closely related murid hosts, *Rhabdomys pumilio* (Sparrman) and *Rhabdomys dilectus* (de Winton), as models, to investigate if between-host species differences in helminth infections are mainly caused by level of sociality (social *R. pumilio* and solitary *R. dilectus*) or environmental conditions (more xeric *R. pumilio* and more mesic *R. dilectus*), (3) investigate the effect of social and spatial behaviour, of *R. pumilio* and *R. dilectus*, on parasite community organization with reference to species co-occurrence and nestedness and (4) explore factors responsible for patterns in similarity in helminth species composition in *R. pumilio* and *R. dilectus*. In total 168433 specimens, comprising 56 helminth taxa were recovered from at least 16 rodent, and sengi and shrew species (n = 1079). The helminth species represented 26 genera of which 16 were nematodes, nine cestodes and one acanthocephalan. Overall, the most abundant helminth species was the nematode *Heligmonina spira* (133.8 ± 13.5), which was also the most prevalent (26.1%). *Rhabdomys dilectus* harboured 19 nematode and 7 cestode species while *R. pumilio* harboured 10 nematode and 5 cestode species. Seven helminth species (4 nematodes and 3 cestodes), were shared between the two rodent species, however, they also

harboured their own specific helminths. In general, monoxenous (direct life cycle) nematodes were present in higher abundance compared to heteroxenous (indirect life cycle) nematodes and cestodes. Several novel helminth-host and helminth-locality records, in addition to several potentially new helminth species were noted. Life cycle-specific geographic distributions were recorded for monoxenous and heteroxenous nematodes. Helminth infections varied spatially and seasonally with significantly higher helminth abundance and prevalence in the months following the wet season. Cestode infection as well as nematode abundance, species richness or prevalence did not differ between *R. dilectus* and *R. pumilio* in between-host species comparisons. However, incidence of nematode infection was significantly higher in *R. dilectus* than in *R. pumilio*. Within-host species comparison showed that nematode abundance and species richness in infracommunities of *R. pumilio* inhabiting the relatively more xeric Karoo biome were significantly lower than in those inhabiting the relatively less xeric Fynbos biome. General patterns of helminth co-occurrence were similar (positive) in the two hosts, but the strength of positive associations increased with an increase in the mean number of helminth species in *R. dilectus* and in prevalence of infection in *R. pumilio*. The two host species differed in the relative frequency of positive and negative pairwise species co-occurrences (only positive in *R. dilectus* and both positive and negative in *R. pumilio*). Nestedness-related patterns in helminth infracommunities were only found in *R. pumilio* (predominantly anti-nested), whereas the opposite was the case for their component communities (only nested in *R. dilectus*). The level of infection was generally associated with the manifestation of non-randomness in helminth assemblages. Although species composition of infracommunities largely overlapped between *R. dilectus* and *R. pumilio* they were still significantly different between the two species. In both rodent species helminth infracommunities were more similar among individuals from the same locality than among localities or biomes. This pattern was more distinct for *R. dilectus*, which may be attributed to larger spatial distribution. Also,

helminth species composition among localities correlated significantly negatively with geographic distance between localities, with a higher rate of decrease of similarity of helminth assemblages with an increase in geographic distance in *R. pumilio* than in *R. dilectus*. It is evident that spatial variation in helminth infections and community structure of helminth assemblage are dependent on a complex interplay of host and parasite related factors, compounded by environmental variation.

Opsomming

Beskrywende inligting vorm die basis vir breër ekologiese vraagstukke en speel 'n belangrike rol in studies wat poog om patrone in parasietverskeidenheid, verspreiding en spesies-samestellings bloot te lê. Inligting oor parasietvoorkoms verskaf basieseinsette vir die opstel van verspreidingskaarte wat waardevol kan wees met die identifisering van siekterisiko en proaktiewe siekte bestuur. Huidige kennis van helmint-gasheer en helmint-lokaliteit assosiasies by kleiner soogdiere in Suid- en suider-Afrika is tans gebrekkig. Daar bestaan dus onsekerheid oor die vormingseffek van gasheer- en omgewingsfaktore op helmint infeksies en gemeenskapstrukture binne ‘n klimatologiese diverse landskap. Ten einde hierdie gebrek van inligting aan te spreek het hierdie studie ten doel: (1) om beskrywende inligting oor helmint-gasheerverhoudings en die ruimtelike en temporale verspreiding van helmintparasiete wat met klein soogdiere geassosieer is, te bepaal, (2) deur die gebruik van twee naverwante knaagdier gashere, *Rhabdomys pumilio* (Sparrman) en *Rhabdomys dilectus* (de Winton), as toonbeelde, te bepaal of tussen-gasheerspesies verskille in helmint infeksies hoofsaaklik veroorsaak word deur sosialiteitsvlak (sosiale *R. pumilio* teenoor enkellopende *R. dilectus*) of verskille in omgewingstoestande (meer xeriese *R. pumilio* teenoor die meer mesiese *R. dilectus*); (3) om die effek van sosiale en ruimtelike gedrag van *R. pumilio* en *R. dilectus* op parasietgemeenskapsorganisasie met verwysing na mede-voorkoms en genestheid, te ondersoek en (4) om faktore verantwoordelik vir ooreenkomspatrone van helmintspesie-samestellings van *R. pumilio* en *R. dilectus* te te verken. Vanuit ten minste 16 spesies van knaagdiere, klaasneusmuise en skeerbekmuise (n = 1079), is 168433 helminte, wat 56 helmint taksa verteenwoordig, versamel. ‘n Totaal van 26 helmint genera, bestaande uit 16 rondewurm, nege lintwurm en een akantokefalied is gevind.

Die helmintspesies met die hoogste voorkoms was die rondewurm *Heligmonina spira* (133.8 ± 13.5), wat dan ook die volopste (26.1%) was. *Rhabdomys dilectus* was besmet met 19 rondewurm en 7 lintwurm spesies, en *R. pumilio*, 10 rondewurm en 5 lintwurm spesies. Sewe helmintspesies (4 rondewurm en 3 lintwurm) het by beide knaagdierspesies voorgekom, maar elk was ook besmet met unieke helmintspesies. Oor die algemeen was monoxenous (direkte lewensiklus) rondewurms teenwoordig in hoër getalle in vergelyking met heteroxenous (indirekte lewensiklus) ronde- en lintwurms. Verskeie nuwe helminh-gasheer- en helmint-lokaliteit rekords, asook verskeie moontlik tot nog toe onbeskryfde en dus vermoedelik nuwe helmintspesies, is aangeteken. Lewenssiklus-spesefieke geografiese verspreidings is gekarteer vir monoxenous en heteroxenous rondewurms. Helmintinfeksies het ruimtelik en seisoenaal verskil met betekenisvolle hoër volopheid en voorkoms gedurende die maande wat volg op die reënvalseisoen. Met tussen-gasheervergelykings het lintwurm asook rondewurm volopheid, spesiesrykheid en voorkoms nie beduidend tussen *R. dilectus* and *R. pumilio* verskil nie. Die insidens of trefwydte van rondewurm infeksie was egter aansienlik meer by *R. dilectus* as by *R. pumilio*. Binne-gasheerspesies vergelykings het getoon dat rondewurm volopheid en spesiesrykheid van helmintinfragemeenskappe beduidend laer was by *R. pumilio* wat die droër Karoo bioom bewoon in vergelyking met dìe woonagtig in die relatief minder droër Fynbos bioom. Algemene patrone vertoon deur helmint mede-voorkomste was eenders (positief) by beide gasheerspesies. Positiewe assosiasies het egter versterk met toename in gemiddelde aantal helmintspesies by *R. dilectus* en met infeksie-volopheid by *R. pumilio*. Die relatiewe frekwensie van positiewe en negatiewe paarsgewyse spesie medevoorkomste (slegs positief by *R. dilectus* en beide postief en negatief by *R. pumilio*) het tussen die twee gasheerspesies verskil. Van die twee gasheerspesies het slegs *R. pumilio* genestheid-verwante patrone van sy helmint infragemeenskappe getoon (hoofsaaklik teen-genestheid), terwyl die teenoorgestelde die geval was by die helmint komponente gemeenskappe (slegs genestheid-verwante patrone

is gevind met *R. dilectus*). Die vlak van helmint infeksie was oor die algemeen geassosieerd met die bevestiging van nie-toevalligheid by die samestelling van helmintversamelings. Samestellings van helmintspesies het grootliks oorvleuel tussen *R. dilectus* and *R. pumilio*, maar het steeds beduidend verskil tussen die twee gasheerspesies. Daar was meer ooreenstemming by beide gasheerspesies in helmint-infragemeenskappe tussen individue vanaf dieselfde lokaliteit as tussen die van verskillende lokaliteite of biome. Diè patroon was dan ook meer beslis so by *R. dilectus*, heel moontlik as gevolg van die gasheerspesies se groter ruimtelike verspreiding. By beide gasheerspesies het tussen-lokaliteit helmintspesie-samestellings beduidend negatief gekorreleer met geografiese afstand tussen lokaliteite, met ‘n hoër tempo van afname van ooreenkomste tussen helmint-samestellings met ‘n toename in geografiese afstand by *R. pumilio* as by *R. dilectus*. Dit is duidelik dat die ruimtelike variasie van helmint infeksies asook gemeenskapstrukture van helmint-samestellings afhanklik is van ‘n komplekse wisselwerking van gasheer- en parasietverwante faktore, met omgewingsvariasie as ‘n bykomende oorsaak.

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Table of Contents

List of Figures

List of Tables

Appendix A: List of supplementary figures

Appendix B: List of supplementary tables

Chapter 1

General introduction

*As regards the authorities of host and parasite genera and species, mentioned in this dissertation, the following approach has been adopted. The authorities of hosts and parasites collected during this study are listed in Tables 2.3 and 2.5, repectively. However, authorities of parasies and hosts that form no part of this study are cited in the text when first mentioned. Some repetition might occur in Chapters 2-5, as these follow a specific journal format.

1. Helminth taxonomy and biology

By definition, parasites are closely associated with their hosts and have co-existed over time. Helminth parasites, of which acanthocephalans, nematodes, cestodes and trematodes are the four major groups, are endoparasites of practically all mammals. Globally, acanthocephalans comprise more than 1200 species, nematodes some 10500 species, cestodes approximately 5000 species and trematodes more than 15000 species (Poulin 2007a). About one third of all described nematode genera are parasites of vertebrates (Anderson 2000).

1.1 Life cycles and resource partitioning

Helminth parasites have diverse life cycles. The majority of nematodes possess a monoxenous strategy (direct life cycle where no intermediate host is required) and infective stages are directly ingested while foraging, while some are heteroxenous (indirect life cycle strategists where an intermediate host is required for the development to the infective stage, which is then ingested by the definitive host). Nematodes therefore are often the most represented in helminth communities of small mammals. A study on the helminth community structure of *Rattus leucopus* (Gray), from Australia, Papua New Guinea and Papua revealed the presence of several families that belong to all six nematode orders (Smales and Spratt 2004). Similar to a study on the helminth species richness of Iberian rodents (Feliú et al. 1997), these included monoxenous representatives (Heligmosomidae, Heligmonellidae), including autoinfection (Oxyuridae), as well as heteroxenous strategists (Ascarididae, Maupasinidae, Subuluridae, Physalopteridae, Rictulariidae and Spiruridae) (Feliú et al. 1997; Anderson 2000; Smales and Spratt 2004). Trematodes, acanthocephalans and cestodes are exclusively heteroxenous, often using arthropods as intermediate hosts, and their species richness and prevalence is thus often higher in small mammal species that mainly include insects as part of their diet. The latter include several rodent species and shrews (Haukisalmi 1989; Novikov 1995; Shimalov 2001;

Kinsella 2007). Cestodes that commonly occur in rodents and insectivores belong to several families, including the Davainaeidae, Dilepididae, Taeniidae and Hymenolepididae, with the latter usually being the most specious (Haukisalmi 1989; Feliú et al. 1997; Shimalov 2001; Ribas and Casanova 2005; López-Darias et al. 2008; Führer et al. 2010; Ondríková et al. 2010).

A further strategy of parasites, aimed to minimise competition and to maximise survival, is to partition the available regions in and on their hosts, often developing distinct ecomorphs that reflect their host resource utilization (Dick 2007; Tello et al. 2008). Competition for microhabitats in and on the host helps drive the evolution of these divergent ecomorphologies resulting in greater species richness (Patterson et al. 2008). The gastrointestinal tract of rodents offers several distinct niches for helminth colonisation, the stomach, small intestine, caecum and colon. From the literature, it is apparent that all these niches are utilised by helminths. Acanthocephalans, trematodes and cestodes appear to be limited to the small intestine. However, nematodes have inconsistent preferences: adults of the Spirurida are usually found in the stomach, those of the Strongylida in the small intestine, and heterakids, subulurids and oxyurids occur in the caecum and/or colon; some members of the Enoplida inhabit the stomach and/or small intestine while others are found in the caecum (Wilamowski et al. 2002; Portolés et al. 2004; Smales and Spratt 2004).

In addition to differences in site preference, helminths have evolved different ways to utilise the resources available at a given site, which enables them to co-exist. Based on the classification of Bush (1990), helminths of rodents, shrews and sengis can be assigned to four different feeding guilds, which irrespective of their systematic position, feed in the same way: the nematode guild is closely associated with the mucosa and actively feeds on tissue and/or lumen contents, while cestodes and acanthocephalans absorb nutrients via their body surface

and can be split into mucosal and luminal absorbers. The trematode guild not only absorbs nutrients over the tegument, but also ingests semi-solids such as blood, mucous or intestinal debris.

1.2 Helminth diversity associated with small mammals

Small mammals such as rodents, shrews and sengis are known to harbour a diverse array of helminth parasites including acanthocephalans, trematodes, cestodes and nematodes, with the latter two taxa being the most common (Collins 1972; Haukisalmi 1989; Wilamowski et al. 2002; Fuentes et al. 2000; Portolés et al. 2004; Smales and Spratt 2004; Feliú et al. 1997, 2009). Approximately 20 nematode genera, comprising 12 families have been recorded from rodents and insectivores in Africa (Ugbomoiko and Obiamiwe 1991; Behnke et al. 2000, 2004; Barnard et al. 2003; Fichet-Calvet et al. 2003; Durette-Desset and Digiani 2005; Brouat et al. 2007; Froeschke et al. 2010). The oxyurid nematodes (*Syphacia* spp., *Dentostomella* spp., *Aspiculuris* spp.) and trichostrongylid nematodes (*Neoheligmonella* spp., *Heligmonina* spp.) are the most prominent, are monoxenous and have a wide host spectrum. In both groups, oral infection through contaminated food is the main transmission route, with self-grooming and allo-grooming, as well as coprophagy increasing transmission rates (Hernandez and Sukhdeo 1995). For *Neoheligmonella* spp., skin penetration has also been established as a possible route of infection (Anderson 2000). In contrast to the two mentioned groups, the spirurids recovered from these small mammals are typically heteroxenous. *Protospirura muricola* and *Subulura* spp. were the most common genera and species recorded in the above-mentioned studies. While nematodes were usually the dominant helminths, a number of cestodes (*Hymenolepis* spp., *Rodentolepis* spp., *Taenia* spp., *Raillietina* spp., *Mesocestoides* spp.) and trematodes (*Echinostoma* spp.), as well as the acanthocephalan genus *Moniliformis* (Behnke et al. 2004) have been recovered from these hosts as well (Ugbomoiko and Obiamiwe 1991; Behnke 2004;

Brouat et al. 2007). All free-living animal species have their own unique parasite species assemblages with the result that there are many more parasite than free-living species in any given system (Fritz 1983). An assemblage of all parasites of different species in a given host individual forms an infracommunity (Pence 1990; Poulin 2007a). The combined infracommunities of the entire host population, at a given locality, is referred to as a component community (Poulin 2007a). The latter seem to vary across spatial and temporal scales, which provides an opportunity to study their patterns and structuring processes at different levels and times (Poulin 1997). Gaining knowledge about parasites may thus offer pragmatic information on the biology, systematics and phylogeny of their hosts and may clarify epidemiological aspects of the transmission of certain diseases (Fritz 1983; Soulsby 1986; Oliva et al. 2008; Matthee et al. 2007).

The biological importance of these small mammal taxa extends further. Several rodent species have adapted well to anthropogenic activities, often being found in close contact to humans and domestic animals, and their helminth fauna can thus pose a disease risk. They have long been identified as integral to the domestic cycle of the zoonotic helminth *Trichinella spiralis* (Owen, 1835), and they are known reservoir hosts for diseases, such as rabies, toxoplasmosis, plague and a range of helminth infections (Soulsby 1965). With regard to the latter, several helminth species for which small mammals act as definitive, intermediate or paratenic hosts have zoonotic potential and can cause diseases with high morbidity if transmitted to humans or domestic animals. These include for example the nematodes *Trichinella* spp., *Toxocara canis* (Werner, 1782) and *Angiostrongylus* spp. (Casanova et al. 2006), the cestodes *Raillietina* spp., *Rodentolepis nana* and the acanthocephalan *Moniliformis moniliformis* (Chaisiri et al. 2015).

2. Factors that shape helminth diversity and community structure

Helminth parasites have a dual environment that includes the environment outside and within the host (definitive and intermediate). Dallas and Presley (2014) hold that parasite distributions within a locality are shaped by a combination of biotic and abiotic factors that form a common environment. It is thus expected that both environmental and host factors will influence the distribution of individual parasite species and populations in space and time.

2.1 Environmental factors: climate and vegetation

Environmental factors such as vegetation and climatic conditions (rainfall, temperature and humidity) have a major influence on the spatial distribution of parasites across the landscape (Stromberg 1997). Two of the most important environmental factors affecting the composition of helminth communities are temperature and rainfall, as they have a direct influence on the survival of the free-living stages (eggs and larvae) of these parasites (Crowe 1977; Mas-Coma et al. 2008). In this context, vegetation cover at a given site can also play an important role (Brouat et al. 2007). The eggs of many helminths found in rodents, such as heterakids, ascaridids, subulurids, physalopterids, are thick shelled and can survive in the environment for extended periods provided that soil moisture conditions are suitably high and temperatures not too extreme; they are, however, highly susceptible to desiccation (see Anderson 2000). In a study on the helminth fauna of the Lusitanian pine vole, *Microtus lusitanicus* (Gerbe), in the Iberian Peninsula, the abundance of *Heligmosomoides laevis* (Dujardin, 1845) and *Taenia tenuicollis* Rudolphi, 1819, amongst others, was shown to be dependent on mean monthly temperature and mean seasonal rainfall (Feliú et al. 2009). Froeschke et al. (2010) investigated the influence of rainfall, relative humidity and temperature on nematode communities of *R. pumilio* along a precipitation gradient from the Cape in South Africa to northern Namibia. Results showed a positive correlation between the infection rates of 15 helminth species and

rainfall and relative humidity, while a negative correlation was recorded with temperature. The study also recorded an association between parasite abundance, prevalence and species richness in this rodent species along a continuous natural climatic gradient (Froeschke et al. 2010). Parasites with heteroxenous life cycles, involving intermediate hosts that could act as buffers against adverse climatic conditions, are thought to be less sensitive than those with monoxenous life cycles (Junker et al. 2008; Monello and Gompper 2011).

The role of climatic conditions is, however, not limited to a direct influence on the survival of free-living parasitic stages. Higher rainfall and moderate climatic regions generally display high plant diversities and, since plant and insect diversity are largely related (Hawkins and Porter 2003), high insect diversities as well. According to Pence (1990), there is a positive relationship between the helminth diversity within a host and habitat heterogeneity. Rodents and insectivores, which forage in complex habitats with high plant diversities and consequently a high insect diversity, might therefore have a higher parasite diversity, especially cestodes (Betterton 1979; Ondríková et al. 2010). Yet, rainfall and moderate temperatures are not the only factors that can lead to a species rich environment, and a number of studies have found that insect diversity in the floristically rich South African Grassland biome was equally rich despite droughts and highly fluctuating temperatures (Procheş and Cowling 2007; Procheş et al. 2009). To date, no comparative data exist regarding the abundance or species richness of helminth assemblages in small mammals in the various biomes in South Africa.

2.2 Host-associated factors

There are various host-related factors that can influence the abundance and prevalence of helminth species within host populations. Several studies have recorded the importance of host size, age, sex, food, habitat preference, reproductive state, host density and sociality (group

size) (Moura et al. 2003; Arneberg 2002; George-Nascimento et al. 2004; Bertola et al. 2005; Dick and Dick 2006; Patterson et al. 2008). In addition, small mammals vary in life history characteristics that include diet (e.g. grass, seeds and invertebrates), behaviour (e.g. sociality) and habitat preference (e.g. vegetation types). From this it is expected that the life history characteristics of small mammals will influence their contact rate with parasites (Anderson 2000; Turner and Getz 2010; Dybing et al. 2013).

Host body mass and size are often associated with greater food intake resulting in greater intake of infective parasitic stages, or with greater available energy, space and/or microhabitats within the host that can support larger and more diverse parasite populations (Marshall 1981, 1982; Guégan and Hugueny 1994, Poulin 1997; Arneberg et al. 1998b; Poulin and George-Nascimento 2007; Patterson et al. 2008). In small mammals, a mixed picture emerges. In Finland, Haukisalmi (1989) attributed heavier helminth burdens recorded in common shrews, *Sorex araneus* Linnaeus, when compared to congeners to, amongst other factors, its larger size. Bjelić-Čabrilo et al. (2009) found a positive if not statistically significant correlation between intensity of infection and size in bank voles, *Myodes glareolus* (Schreber) [syn. *Clethrionomys glareolus* (Schreber)], in Serbia. However, no relationship between host size and species richness was found in a comparative study on Iberian rodents (Feliú et al. 1997) nor between the prevalence of *Calodium hepaticum* (Bancroft, 1893) infection and the body weight (and age and sex) of mice from human inhabited houses from the Azores archipelago in Portugal (Resendes et al. 2009).

Host age may have several influences on helminth communities. Since many helminths are long-lived, host ageing can be seen as an increased exposure to infective stages over time, thus allowing for an accumulative effect on species richness and intensity of infection (Behnke et

al. 2000, 2008; Ondríková et al. 2010). On the other hand, younger animals might be more susceptible to the establishment of parasites, as their immune system is less efficient than that of older animals (Soulsby 1969). Several studies on ectoparasites concur that older hosts harbour higher parasite loads and increased species abundance (Overal 1980; Komeno and Linhares 1999; Bertola et al. 2005; Dick and Dick 2006). However, limited data exist for endoparasites (Davies et al. 2008). In studies on spiny mice, *Acomys dimidiatus* Cretzschmar, and wood mice, *Apodemus sylvaticus* Linnaeus, respectively, Behnke et al. (2000, 2005) consider host age as perhaps the most important intrinsic factor that contributes to helminth species richness and abundances (younger animals generally harbouring fewer species of helminths and having lower worm burdens). However, in another study on helminth communities of spiny mice, none of the monoxenous oxyuroid nematodes showed significant variation in abundance between host sex or age classes (Behnke et al. 2004). Pence (1990) suggested that the structure and dynamics of helminth communities in mammal populations at specific localities, at the component community level, are most affected by changes in host age over seasons. When assessing temporal and spatial effects in helminth communities of bank voles in three woodland sites, Behnke et al. (2008) concluded that, for species richness and diversity, most deviance was accounted for by host age while sampling site accounted for most of the deviance related to prevalence and abundance of infection. Therefore, the effect of host age on helminth communities appears to be variable with host species, immune status, season and site interactions.

Some ecological theories reasoned that males should be more prone to parasite infection because of a number of factors, such as high vagility, larger size and testosterone influenced immune suppression (Folstad and Karter 1992; Arneberg 2002; Moore and Wilson 2002; Wirsing et al. 2007). Lately, several studies have confirmed that males do have higher parasite

prevalences and intensities (Moore and Wilson 2002; Ferrari et al. 2004). Schalk and Forbes (1997), however, demonstrated that while such a male bias existed for ectoparasites in mammalian hosts, no such bias was obvious for helminth parasites. With regard to small mammals there are inconsistent reports. Neither Behnke et al. (2000) nor Ribas and Casanova (2005) or Milazzo et al. (2002) found differences between the two sexes concerning prevalence and infection intensity in their studies on helminths of *A. dimidiatus, Talpa europaea* Linnaeus and *Talpa romana* Thomas, respectively. Conversely, two studies reported on differences between sexes in *T. europaea* and *Talpa occidentalis* Cabrera and these were attributed to sexual dimorphism and the high mobility of males within their environment (Prokopič and Grulich 1976; Casanova et al. 1996 both in Ribas and Casanova 2005). Thus, it would appear that in small mammals a gender related influence on helminth prevalence or intensities could be expected in especially those hosts where there is pronounced sexual dimorphism, i.e. differences in size, or where males and females display behavioural differences.

It would appear that densely populated host communities, resulting in a high degree of interaction, would have higher endoparasite densities and/or diversities. In a study on the communities of monoxenous gastrointestinal helminths of the order Strongylida in mammalian hosts, Arneberg (2002) found a strong positive correlation between host population density and species richness. Similarly, host density was also regarded as an important factor facilitating high parasite species richness for helminth species that have monoxenous and heteroxenous life cycles in terrestrial mammals (Morand and Poulin 1998). Both studies thus confirm the theory that higher host densities lead to a higher probability of host exposure to infective stages of different parasites and consequently to increased transmission rates. In a study investigating the relationship between bobwhite quail, *Colinus virginanus* Linnaeus, group-size and

intestinal helminths, Moore et al. (1988) found that helminth burdens increased with group-size, and helminths with rapid, direct life cycles showed the most pronounced effect.

A frequently and regularly performed behavioural pattern of rodents is grooming, which, according to Hart (1990, 1992), enables animals to avoid or minimize their exposure to parasites. While grooming may be beneficial in reducing ectoparasite burdens in rodents (e.g. Shaw et al. 2003), it can promote infection with especially oxyurid (Bajer et al. 2005) and trichostrongylid endoparasites (Hernandez and Sukhdeo 1995). Gravid females of many members of the Oxyuroidea migrate to the anus of the host and deposit eggs in the perianal region, where they rapidly complete their development to the infective stage. In most cases, the eggs are then readily transferred from the perianal region to the host's mouth by grooming activities (see Anderson 2000). In a study on bank voles in Poland, autoinfection with *Syphacia* spp. commonly occurred when voles cleaned their fur (Bajer et al. 2005). In some instances, eggs are deposited in the off-host environment (e.g. nests), where they can survive for extended periods and remain infective under favourable environmental conditions. Coprophagia is another host trait that may increase transmission, as eggs can easily adhere to faeces and are readily transmitted to coprophagic hosts such as lagomorphs and rodents (Anderson 2000).

Numerous studies have demonstrated a relationship between the host's feeding habits and helminth community structure. The type of food consumed by the host (or diet) will influence the rate of contact with infective stages. For example, a diet containing a higher proportion of possible intermediate hosts (e.g. arthropods and other invertebrates) will result in a higher infection of heteroxenous helminths (Pence 1990; Ondríková et al. 2010). In general, rodents feed on plant material, seeds and to a lesser extent on invertebrates (Perrin 1981; Skinner and Chimimba 2005; Kingdon et al. 2013). Shrews are regarded as opportunistic carnivores, living

entirely on a range of invertebrates that include mainly insects, but also snails and slugs (Langer 2002; Skinner and Chimimba 2005). The diet of sengis mainly comprises of invertebrates (90%) of which the majority are isopterans and formicids, followed by coleopterans and other arthropods (Skinner and Chimimba 2005). Haukisalmi (1989) found 23 helminth species in three shrew species (*Sorex* spp.) from Finland. He concluded that one of the shrew species, *S. araneus*, harbours heavier helminth burdens due to its larger size, great abundance and extensive diet, which includes numerous arthropod species. Similarly, Ondríková et al. (2010) contributed a higher species diversity seen in helminth communities of *Apodemus agrarius* Pallas, when compared to *Apodemus flavicollis* Melchior, to the higher proportion of invertebrates in the former's diet. Similarly, the primarlily herbivourous diet of bank voles in Serbia may explain higher monoxenous nematode infections (Bjelić-Čabrilo et al. 2009).

Host habitat preference brings into play many of the factors discussed above, such as environmental conditions, vegetation cover and habitat heterogeneity, as well as associated arthropod diversity. Hosts whose habitat requirements coincide with conditions that are favourable for the completion of parasitic life cycles, such as adequate temperature and humidity, are more likely to be exposed to higher burdens of helminths (Pence 1990; Behnke et al. 2000; Feliú et al. 2009). Host species that occur in heterogenous habitats, or hosts that are able to utilise a wide range of different habitat types, may have a higher probability to be exposed to possible intermediate hosts of a number of heteroxenous helminths. Furthermore, they would more than likely come into contact with helminths of a larger variety of other host species sharing their habitat. All of the above might result in higher species richness as well as infection intensity of their helminth assemblages (Betterton 1979; Brouat et al. 2007).

2.3 Parasite-associated factors

Several parasite-associated factors can influence the abundance and distribution of helminth parasites. These include life cycle, mode of transmission and host range (e.g. level of host specificity). As mentioned above, nematodes have monoxenous and heteroxenous life cycles while cestodes and acanthocephalans are heteroxenous (Anderson 2000). The free-living infective stage of monoxenous nematodes are more densely distributed, and thus available to hosts, in the environment compared to heteroxenous taxa that are more widely distributed and in lower densities (Anderson 2000; Turner and Getz 2010; Dybing et al. 2013). This is mainly due to the fact that heteroxenous taxa require intermediate hosts as part of their life cycle.

Parasites differ markedly in their degree of host specificity (Holmes and Price 1980) and may be divided into two major categories: specialists and generalists (Margolis and Arthur 1979; Holmes and Price 1980). In their comparative analysis of species richness of Iberian rodents, Feliú et al. (1997), divided helminths into four categories, following Euzet and Combes (1980, in Feliú et al. 1997): oioxenous parasites specific to a given host species, e.g. *Syphacia frederici* Roman, 1945, stenoxenous parasites specific on generic level, e.g. *Syphacia obvelata* (Rudolphi, 1802), oligoxenous parasites limited to a certain host family, e.g. *Trichuris muris*, and euryxenous parasites that can utilise a wide range of unrelated hosts, e.g. *Mastophorus muris*. Stenoxenous and oioxenous parasites were by far in the minority and the majority of helminths fell in either the oligoxenous or euryxenous category (Feliú et al. 1997, 2009). However, specific ecological factors that influence the degree to which parasites are host specific are difficult to determine (Krasnov et al. 2004a, 2005c, 2006a,b). Poulin (2007a) suggests that a decrease in host specificity occurs where host behaviour exposes a parasite to a variety of host species to which transmission may be favourable. Furthermore, the recruitment by and establishment of a parasite species in a host is governed by host phylogeny and

evolutionary adaptation between host and parasite, thus, phylogenetically closely related hosts are likely to harbour the same specialist parasite species (Holmes 1990). However, co-evolution is not the only factor determining if a certain host is suitable for a certain parasite. If parasite survival in a given host depends on one or several biological host characteristics, then other host taxa, even unrelated ones, but sharing the same combination of traits, may serve as host. This concept of ecological fitting allows even specialised parasites to switch hosts and to capitalise on chance exposure to new hosts, even if there is no history of co-evolution between the parasite and this host (Wilkinson 2004; Brooks et al. 2006). Hence, it might not be surprising that co-occurring host species with similar diets and habitats may harbour similar or even identical parasite faunas even when hosts are phylogenetically unrelated. For example, Holmes (1990) concluded that most of the helminths that live in the alimentary tract of various unrelated marine fish have a relatively broad host spectrum and exhibit weak phylogenetic patterns in their distributions. Similar results were recorded by Lile (1998), and it was suggested that host ecology, such as habitat use, rather than the phylogenetic background influences the appearance and establishment of helminth faunas in flatfish species in northern Norway.

3. Effects of parasites on their hosts

Parasites can contribute significantly to population fluctuation in many host species and the effects on dominant or keystone species can have far reaching effects on ecosystem processes (Loreau et al. 2005). The negative impact of parasites is closely linked to the considerable strain they put on host resources (Kuris et al. 2008). The mechanisms used by hosts to resist and reduce parasite infections are especially energetically costly and valuable resources are invested in physiological and behavioural traits for their detection, prevention and response (Rigby et al. 2002). The immune system, in particular, continually drains energy and resources

for, amongst others, the proliferation and movement of cells (Esch et al. 1975). Empirical evidence is provided in various studies, which include the study by Scott (1987), where it was recorded that host numbers increased concomitantly with a reduction in the transmission rate of the nematode *Heligmosomoides polygyrus* (Dujardin, 1845) as well as its elimination from the CD1 Swiss mice population. Similarly, Hudson et al. (1998) found that by experimentally reducing parasite burdens in free-ranging red grouse, *Lagopus lagopus scoticus* Linnaeus, an improvement was noted in the breeding success of the grouse. More recently, Eira et al. (2007) recorded a relationship between high burdens of the relatively large cestodes, *Mosgovoyia ctenoides* (Railliet, 1890) and the body condition in European rabbits, *Oryctolagus cuniculus* Linnaeus. By implication, the effect of parasites on the fitness of host populations could impact not only on the abundance of individual host species, but also on interactions among competing (co-occurring) host species, and thus on the structure of host communities (Scott 1987).

4. Physio-climatic characteristics of the biomes in South Africa

The physical position (e.g. southern tip of Africa and surrounded by two oceans), topography and climate regimes of South Africa have contributed to the formation and establishement of diverse vegetation types. Nine biomes are recognised in South Africa of which six cover most of the country (Mucina and Rutherford 2006). The latter include the Savanna, Grassland, Thicket, Fynbos, Succulent- and Nama Karoo.

The Savanna biome comprises the southernmost extension of the largest biome in Africa, covering over one-third of South Africa. It is characterized by a grass ground layer and a distinct upper layer of woody plants. Vegetation structure varies extensively, dependent on precipitation and altitude: in the more xeric areas of this biome the upper layer is at ground level and the vegetation is referred to as shrub veld; in mesic areas (north-eastern South Africa)

as woodland, whereas the intermediate stages are referred to as bushveld. The environmental factors delimiting this biome are complex: precipitation varies from 235 to 1000 mm per annum; altitude ranges from sea level to 2000 m; frost may be absent or occur for up to 120 days per year; and almost every major geological and soil type occurs within the biome. Dominance of grass species is dependent on summer rainfall and the lack thereof is a major factor delimiting this biome. Almost all plant species are adapted to fire survival (Rutherford and Westfall 1986, Mucina and Rutherford 2006).

The Grassland biome is found chiefly on the high central plateau of South Africa, and the inland areas of KwaZulu-Natal and the Eastern Cape. Altitude varies from near sea level to 2850 m above sea level. Grasslands are dominated by a single layer of grasses, the amount of cover being dependent on precipitation. Trees are absent, except in a few localized habitats while geophytes are often abundant. The Grassland biome is considered to have an extremely high biodiversity, second only to that of the Fynbos Biome (Rutherford and Westfall 1986).

No formal ‘Thicket biome’ is recognized in the scientific literature. However, where rainfall is deficient, the vegetation that replaces forest does not comply with that of typical ‘Forest’, not attaining the required height or the many strata below the canopy. The vegetation types within the ‘Thicket biome’ share floristic components with almost all the formal biomes (Cowling 1984; Everard 1987).

The Fynbos biome, within the Cape Floristic region, refers to two key vegetation groups (Fynbos and Renosterveld) within the winter rainfall area at the southern tip of South Africa. The Cape Floristic region is considered to be essentially Fynbos due to the overwhelming contribution of Fynbos vegetation to species richness and endemicity in the region. The biome

is characterized by a high plant species richness and high endemicity (68% of plant species are confined to the Cape Floral Kingdom). Over 7000 of the plant species occur in only five Fynbos vegetation types, with some 1000 additional species in three Renosterveld vegetation types. Thus, the Fynbos biome contains most of the floral diversity. Fynbos must burn, but fires in spring, instead of late summer, or too frequent fires (preventing the plants to seed) could well eliminate species (Bond and Goldblatt 1984; Cowling 1992; Rebello 1994).

Most of the Succulent Karoo biome covers a flat to gently undulating plain, with some hilly and 'broken' veld, mostly situated to the west and south of the escarpment, and north of the Cape Fold Belt. It occurs at altitudes mostly below 800 m. The extent of the biome is primarily determined by the presence of low winter rainfall (between 20 mm – 290 mm per annum) and extreme summer aridity. The vegetation is dominated by dwarf, succulent shrubs and grasses are rare. The number of plant species, mostly succulents, is very high and unparalleled elsewhere in the world for an arid area of this size (Cowling et al. 1986).

The Nama Karoo biome occurs on the central plateau of the western half of South Africa. Most of the biome is at an altitude of between 1000 and 1400 m. The distribution of this biome is determined primarily by precipitation, which occurs in summer and varies between 100 and 520 mm per year. The dominant vegetation is grassy, dwarf shrubland with grasses being more common in depressions and on sandy soils (Cowling et al. 1986).

5. Small mammal diversity in South Africa

Small mammals such as rodents, shrews and sengis are amongst the most numerous of all mammals, the former accounting for over half of today's mammalian species (de Graaff 1981). Worldwide the Muridae represent the largest family of mammals (Skinner and Chimimba

2005). More specific to South Africa, the Muridae comprise some 50 species, the Soricidae (shrews) 16 species and the Macroscelididae (sengis) seven species (Skinner and Chimimba 2005; Stuart and Stuart 2007; Rathbun 2009). They form part of nearly all terrestrial communities. This is largely due to their high adaptability to a wide range of environmental challenges (de Graaff 1981; Skinner and Smithers 1990). Their large numbers and short generation intervals contribute significantly to the energy flow in nature and make them an important factor in food webs and maintaining ecosystems (Dickman 1999; Bjelić-Čabrilo et al. 2009) and, in South Africa, as in other parts of the world, these small mammal taxa contribute significantly to biodiversity.

The rodent genus *Rhabdomys* Thomas is endemic to southern Africa, widespread and locally abundant and presents an excellent model to investigate the role of host ecology and behaviour as well as environmental conditions on helminth assemblages between closely related hosts. Two morphologically similar species, *R. pumilio* Sparrman and *R. dilectus* De Winton, with an estimated divergence time of ca. 3 Ma are recognised (Rambau et al. 2003). The two species differ in sociality, habitat preference and geographic distribution. *Rhabdomys pumilio* forms social groups and occurs in the winter-rainfall xeric western region of South Africa (Schradin and Pillay 2005) whereas *R. dilectus* is a solitary species and occurs in the mesic eastern regions of South Africa (Dufour et al. 2015). Both species are opportunistic omnivores (Perrin and Curtis 1980; Skinner and Chimimba 2005).

6. Helminth research in Africa with emphasis on South Africa

Historically, research focused on parasite taxa of medical and veterinary importance, and this was especially true for helminths (see Soulsby 1986). Despite the ecological and economic importance of their hosts, and despite the fact that parasites in turn can have a significant impact

on the general fitness of a host individual and the population as a whole (Scott 1987; Hudson et al. 1998; Loreau et al. 2005; Mas-Coma et al. 2008), our knowledge of the helminth communities of rodents and insectivores is scant. This is not only true for South Africa, but the entire African continent. The majority of studies have been conducted in the Palaearctic region (Haukisalmi 1989; Shimalov 2001; Milazzo et al. 2002; Fuentes et al. 2005a,b), while few comprehensive studies address helminth assemblages of small mammals in the Afrotropical region (Behnke et al. 2000, 2004; Fichet-Calvet et al. 2003; Barnard et al. 2003; Brouat et al. 2007), the latter mainly in North and West Africa. In South Africa, current information is limited and known studies are largely restricted to taxonomic records (Ortlepp 1939; Collins 1972), or focus on selected host species such as the eastern rock sengi (*Elephantulus myurus* Thomas and Schwann) in South Africa (Lutermann et al. 2015). Only recently has research been initiated on the diversity and ecology of nematodes and cestodes associated with the rodent genus *Rhabdomys* across South Africa and parts of Namibia (Froeschke et al. 2010, 2013; Froeschke and Matthee 2014). From these studies, it is evident that *Rhabdomys* species harbour both nematode and cestode species, and that parasite abundance and species richness display spatial variation in these rodents. These studies include an investigation into the effect of a rainfall gradient on the helminth species richness of the four-striped mouse, *Rhabdomys pumilio*, in South Africa (Froeschke et al. 2010), conducted along the arid western side of South Africa. At present, however, descriptive information on the host range, geographic distribution, helminth assemblage composition and processes that govern their specific patterns in these small mammals in South Africa are mostly absent.

To date, few studies have attempted to address this lack of insight into the processes governing helminth communities in small mammals in Africa. Apart from the study by Froeschke et al. (2010) there has been a few other studies in Nigeria (Ugbomoiko and Obiamiwe 1991), Egypt

(Behnke et al. 2000, 2004; Barnard et al. 2003,), Senegal (Brouat et al. 2007) and South Africa (Lutermann et al. 2015). These studies emphasised the role of host factors such as age, diet and social behaviour, as well as habitat characteristics on helminth prevalence, abundance and species richness. Two further studies concentrated on hymenolepidid cestodes of the shrew genus *Crocidura* Wagler in Nigeria (George et al. 1990) and the cestode *Raillietina trapezoides* in the fat sand rat, *Psammomys obesus* Cretzschmar, in Tunisia (Fichet-Calvet et al. 2003), respectively.

7. Aims and objectives

Firstly, in order to address the scant information available on helminth communities associated with small mammals, rodents in particular, in South Africa, a countrywide study was initiated to record their fauna and host range, provide baseline data on the spatial distribution of these parasites in multiple vegetation types and, using the rodent genus *Rhabdomys* as model host, establish the temporal variation in species richness, mean abundance and prevalence of nematode and cestode species in climatically distinct regions.

Secondly, in order to establish if between-species differences in helminth infection were mainly caused by difference in sociality or difference in environmental conditions of their respective habitats, patterns of helminth infection were investigated in the two closely related rodents (social *R. pumilio* occurring mainly in xeric habitats and solitary *R. dilectus* occurring mainly in mesic habitats).

Thirdly, to understand the effect of social and spatial behaviour of a host on parasite community organisation, species co-occurrence and nestedness of assemblages of gastrointestinal helminths were studied in the solitary and mobile *R. dilectus* and the social and territorially

conservative *R. pumilio*, to establish whether helminth communities of the two hosts are characterized by a non-random pattern and whether the occurrence or degree of this non-randomness (a) differs between hosts and (b) is associated with abundance, prevalence and diversity of helminths.

Fourthly, to reveal factors responsible for spatial variation in parasite community composition, patterns of similarity in helminth species composition were studied in these two rodent host species that differ in their social and spatial behaviour, living under different environmental conditions. It was questioned (a) whether the hosts harbour similar assemblages and whether these are more dissimilar between than within hosts and (b) whether host social structure, behaviour or environment affects patterns of similarity in helminth species composition of their infracommunities within and among localities. We also investigated whether similarity in species composition of helminth component communities decreases with an increase of geographic distance between host populations and, if so, whether host spatial behaviour affects this decrease.

Specific predictions

Aim 1 - The association of helminths with small mammals in South Africa is presently a poorly studied field. In the descriptive study, it was therefore predicted that a) several new parasite-host associations and b) parasite-locality records would be established. In addition, the possibility of collecting as yet undescribed helminth species was anticipated.

Aim 2 - It was predicted that, if the difference in social structure between the two host species results in differences in patterns of helminth infection, then the effect of sociality would be manifested in the group-living *R. pumilio* exhibiting lower helminth species diversity but

higher parasite burdens compared to the solitary *R. dilectus*. Higher levels of helminth infection were also expected in (a) males than females and (b) reproductively active than in non-reproductive individuals. In analysing the difference in helminth burdens of *R. pumilio* inhabiting the Fynbos and Succulent Karoo biomes, it was predicted that *R. pumilio* living in the Fynbos would have higher helminth infection levels compared to those in the Succulent Karoo.

Aim 3 - In the investigation of non-randomness of helminth community patterns, it was predicted that helminth infracommunities of *R. pumilio* would be more likely to display nested patterns than those of *R. dilectus* due to the group-living nature of the former. It was also expected that nestedness of component communities of helminths will be manifested (a) more strongly than that of their infracommunities and (b) similarly in both host species.

Aim 4 - Investigation of similarity in helminth community structure involved the effect of the respective social and spatial behaviour of the two host species. Here we predicted that the effect of social behaviour will be manifested in the difference between within- and among-localities similarities in the infracommunity species composition being greater in social *R. pumilio* than in solitary *R. dilectus*. Alternately, the effect of spatial behaviour will be manifested in this difference being higher in more mobile *R. dilectus* than in less mobile *R. pumilio*. It was also predicted that the rate of distance decay of similarity will be higher in the more territorially conservative *R. pumilio* than the more mobile *R. dilectus*.

Chapter 2

Helminth parasites of small mammals in South Africa: spatial distribution, host-range and the consequence of season

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Introduction

Small mammals are amongst the most numerous of all mammals, accounting for over half of the currently known mammalian species (Wilson and Reeder 2005). They form part of nearly all terrestrial communities, largely due to their high adaptability to a wide range of environmental conditions (Stoddard 2012). Small mammals vary in life history characteristics that include diet (e.g. grass, seeds and invertebrates), behaviour (e.g. sociality) and geographic range. From this, it is expected that the life history characteristics of small mammals will influence their contact rate with parasites when moving through the vegetation or during feeding (Anderson 2000; Turner and Getz 2010; Dybing et al. 2013).

Small mammals such as rodents, shrews and sengis are known to harbour a diverse array of helminth parasites including acanthocephalans, trematodes, cestodes and nematodes, with the latter two taxa being the most common (Collins 1972; Haukisalmi 1989; Wilamowski et al. 2002; Fuentes et al. 2000; Portolés et al. 2004; Smales and Spratt 2004; Feliú et al. 1997, 2009). Globally, acanthocephalans comprise more than 1200 species, parasitic nematodes some 10500 species, cestodes approximately 5000 species and trematodes more than 15000 species (Poulin 2007a). Several helminth species for which small mammals act as definitive, intermediate or paratenic hosts have zoonotic potential and can cause diseases with high morbidity if transmitted to humans or domestic animals. These include for example the nematodes *Trichinella* spp., *Toxocara canis* Werner, 1782 and *Angiostrongylus* spp. (Casanova et al. 2006), the cestodes *Raillietina* spp., *Rodentolepis nana* (von Siebold, 1852) and the acanthocephalan *Moniliformis moniliformis* (Bremser, 1811) (Chaisiri et al. 2015). Nematodes are represented by two main life history strategies, a monoxenous life cycle, which involves a single host individual, and a heteroxenous life cycle that, in addition to the final host, requires one or more intermediate hosts (e.g. arthropod) for its successful completion (Anderson 2000),

while cestodes, trematodes and acanthocephalans are exclusively heteroxenous (Georgiev et al. 2006; Ribas and Casanova 2006).

Internal parasites have a dual environment that includes the environment within the host (definitive and intermediate) and the environment outside the host. It is thus expected that both host and environmental factors will influence the distribution of individual species and populations in space and time. Aside from physiological and immunological characteristics of hosts affecting parasite-host interaction, there are other more generalised host properties that may impact on parasite communities. These include host geographic range, host density and host body size (Price 1990). For example, studies have found that parasite and particularly helminth species richness is positively related to host geographic range (Price 1980; Price and Clancy 1983). Dallas and Presley (2014) hold that parasite distributions within a locality are shaped by a combination of biotic and abiotic factors that form a common environment. Therefore, environmental factors such as vegetation and climatic conditions (rainfall, temperature and humidity) may influence the spatial distribution of parasites as much as host factors (Stromberg 1997). For example, the presence of nematode and cestode species in red foxes [*Vulpes vulpes* (Linnaeus)] from Western Australia was positively related to average relative humidity, long-term average minimum temperature and the percentage cover of native vegetation (Dybing et al. 2013). In addition, helminth abundance seems to be positively associated with rainfall and precipitation (Froeschke et al. 2010; Pakdeenarong et al. 2014). Though climatic conditions can directly affect the abundance and distribution of helminth parasites (by impacting the survival of free-living egg and larval stages), they can also have an indirect effect through influencing (adversely or favourably) the abundance and distribution of intermediate hosts in any specific area (Dobson and Carper 1992; Anderson 2000). The intrinsic properties of a parasite (e.g. mode of transmission, susceptibility to desiccation and

host range) add another level of complexity. As mentioned above, there is often a positive relationship between parasite and host geographic range (Price 1980). However, host species range (i.e. level of host specificity) can also influence a parasite's geographic range with generalist parasites having larger ranges and higher abundances (Krasnov et al., 2004a; Poulin et al. 2011a) compared to specialist species (Price and Clancy 1983; Gregory 1990; Poulin 2007b). From the above it is evident that empirical data on the host association and distribution of helminth parasites, in space and time, are vital for any comprehensive ecological study. In addition, updated parasite-host lists and parasite distribution maps are useful in demarcating areas at risk of potential zoonoses and aid in timeous implementation of disease management.

To date, the majority of studies on the helminth diversity associated with small mammals have been conducted in the Palaearctic region (Haukisalmi 1989; Shimalov 2001; Milazzo et al. 2002; Fuentes et al. 2005a, b). In the Afrotropical region, less is known with only a few comprehensive studies conducted in North and West Africa (Behnke et al. 2000, 2004; Fichet-Calvet et al. 2003; Barnard et al. 2003; Brouat et al. 2007). In Africa, current information is limited and studies documenting the helminth fauna of small mammals are restricted to incidental observations of a taxonomic nature (Ortlepp 1939; Collins 1972), or focus on single-species and single-locality investigations (Froeschke et al. 2013; Froeschke and Matthee 2014; Lutermann et al. 2014, 2015). More recently, research has been initiated on the diversity and ecology of helminths associated with the rodent genus *Rhabdomys* Thomas across South Africa and parts of Namibia (Froeschke et al. 2010; Spickett et al. 2017). From these studies, it is evident that *Rhabdomys* species harbour both nematode and cestode species, and that parasite abundance and species richness display spatial variation across the landscape. As yet, however, descriptive information on the host spectra and geographic distribution of helminth parasites associated with rodents, shrews and sengis in South and southern Africa is mostly absent. The

region has a rich diversity of small mammal species representing several families of small mammals (Skinner and Chimimba 2005). In South Africa, the Muridae is represented by some 50 species, Soricidae (shrews) by 16 species and the Macroscelididae (sengis) by seven species (Skinner and Chimimba 2005; Stuart and Stuart 2007; Rathbun 2009). Further, South Africa has diverse rainfall regimes (e.g. summer vs winter rainfall) and vegetation types (e.g. grassland vs sparse shrub vegetation) and it is expected that parasite distribution and mean abundances will vary spatially and temporally.

To address the paucity of information we embarked on a countrywide study in South Africa. The aims of the study were to: 1) record the species and host range of helminths that are associated with rodents, shrews and sengis. Current information is restricted to isolated studies (see Verster 1960; Durette-Desset and Digiani 2005; Froeschke et al. 2010; Froeschke et al. 2013; Lutermann et al. 2014, 2015); 2) provide baseline data on the spatial distribution of nematode and cestode species that are associated with these hosts in multiple vegetation types across South Africa, and 3) using the rodent genus *Rhabdomys* as model host, record the temporal variation in species richness, mean abundance and prevalence of nematode and cestode species in climatically distinct regions. Presently there is limited spatial information available for a few helminth species (Ortlepp 1939; Verster 1960; Froeschke et al. 2010) and as a consequence of the generally scant information available on helminths of rodents, shrews and sengis in South Africa, detailed data on possible seasonal effects are minimal.

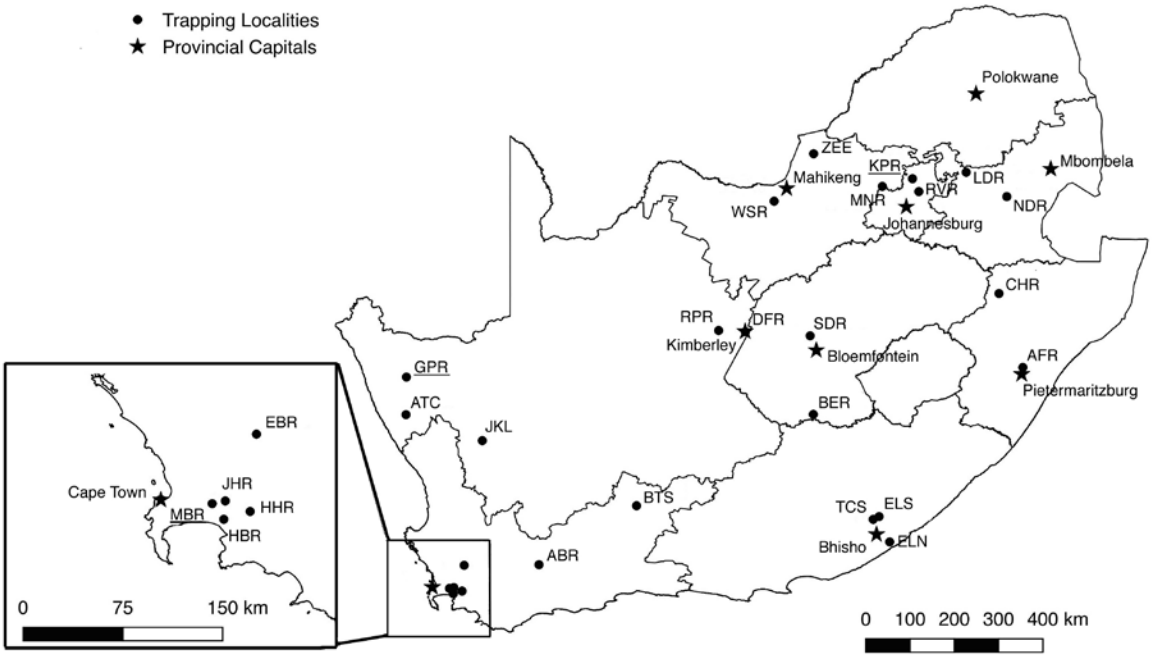


Fig. 2.1 Sampling localities (n = 26) for small mammals in South Africa. Matjiesfontein (ABR); Pietermaritzburg (AFR); Garies (ATC); Bethulie region (BER); Beaufort West (BTS); Newcastle (CHR); Kimberley site 1 (DFR); Wellington (EBR); East London (ELN); Stutterheim site 1 (ELS); Springbok (GPR); Somerset West (HBR); Stellenbosch site 1 (HHR); Stellenbosch site 2 (JHR); Loeriesfontein (JKL); Pretoria site 1 (KPR); Middelburg-Mpumalanga (LDR); Stellenbosch site 3 (MBR); Mooinooi (MNR); Carolina (NDR); Kimberley site 2 (RPR); Pretoria site 2 (RVR); Bloemfontein (SDR); Stutterheim site 2 (TCS); Mahikeng (WSR); Zeerust (ZEE). The three underlined localities (GPR, MBR and KPR) were sampled seasonally (every three months).

Materials and methods

Study area, host collection and parasite recovery

Rodents, shrews and sengis were trapped at 26 localities across South Africa during 2004-2014 (Fig. 2.1, Table 2.1). The main trap season was spring-summer, which is the season when small mammals are more abundant and active (Skinner and Chimimba 2005). In addition, *Rhabdomys* spp. were trapped every three months (seasonally) at three climatically distinct localities (*Rhabdomys pumilio* at GPR, MBR and *R. dilectus* at KPR) (Fig. 2.1, Tables 2.1 and 2.2). The two morphologically similar species, *R. pumilio* and *R. dilectus* have a recent

separation with an estimated divergence time of ca. 3 Ma (Rambau et al. 2003). However, the two species differ in geographic distribution: *R. pumilio* occurs in the more arid western part while *R. dilectus* occurs in the more mesic eastern part of South Africa (Schradin 2005; Schradin and Pillay 2005a).

A standardised sampling design was followed throughout. Animals were trapped using Sherman-type live traps, baited with a peanut butter - oats mixture. Traps were set out in three to five lines (50-100 m length) with 10 m distance between traps and inspected twice per day (morning and afternoon). During the summer months traps were closed during the heat of the day, while the process was reversed in winter when traps were closed during the night. This was done to prevent any trap-related deaths due to adverse environmental conditions. Traps were set for five to seven consecutive days at each locality. Trapped animals were identified using morphological descriptions (Skinner and Chimimba 2005; Stuart and Stuart 2007) and represented rodents (Muridae), sengis (Macroscelididae) and shrews (Soricidae); the latter are morphologically difficult to distinguish at species level and were therefore grouped together. In all cases, only adult animals were targeted and all visibly juvenile animals (see Skinner and Chimimba 2005), were identified, recorded and released at the capture site. Target animals were removed from the traps, placed in plastic bags and immediately euthanized by intraperitoneal injection with Sodium Pentobarbitone (200 mg/kg).

Table 2.1 Sampling localities (n = 26) for small mammals in South Africa. Code (reference code), GPS SOUTH (GPS Co-Ordinates South), GPS EAST (GPS Co-Ordinates East), N (number of host individuals collected), SSR (small mammal species richness), INF (number of infected host individuals) and HSR (helminth species richness).

Nearest town to locality	Code	GPS SOUTH	GPS EAST	N	SSR	INF	HSR
Matjiesfontein	ABR	33.467	20.584	27	1	19	6
Pietermaritzburg	AFR	29.465	30.405	26	1	22	5
Garies	ATC	30.423	17.884	27	1	14	4
Bethulie	BER	30.418	26.152	19	2	16	6
Beaufort West	BTS	32.271	22.565	16	2	16	3
Newcastle	CHR	27.964	29.922	23	1	23	12
Kimberley site 1	DFR	28.742	24.772	32	1	20	4
Wellington	EBR	33.480	19.065	32	1	30	4
East London	ELN	33.005	27.703	22	1	21	5
Stutterheim site 1	ELS	32.490	27.489	38	2	34	8
Springbok	GPR	29.660	17.893	120	1	16	2
summer				30	1	2	1
autumn				31	1	3	1
winter				31	1	2	2
spring				28	1	9	2
Somerset West	HBR	34.055	18.844	34	1	27	4
Stellenbosch site 1	HHR	34.002	19.022	42	1	37	4
Stellenbosch site 2	JHR	33.931	18.854	40	1	40	5
Loeriesfontein	JKL	30.950	19.433	32	2	29	4
Pretoria site 1	KPR	25.635	28.167	158	6	108	15
summer				55	4*	40	11
autumn				60	3*	40	7
winter				30	3	20	4
spring				13	4*	9	8
Middelburg-Mpumalanga	LDR	25.506	29.257	9	6	5	7
Stellenbosch site 3	MBR	33.949	18.766	118	1	102	6
summer				28	1	27	6
autumn				34	1	33	5
winter				31	1	17	5
spring				25	1	25	5
Mooinooi	MNR	25.791	27.553	63	6	37	9
Carolina	NDR	26.000	30.080	10	3	8	8
Kimberley site 2	RPR	28.716	24.230	49	5	14	9

Pretoria site 2	RVR	25.897	28.294	53	4	33	10
Bloemfontein	SDR	28.823	26.089	19	1	8	5
Stutterheim site 2	TCS	32.547	27.368	40	3	32	11
Mahikeng	WSR	26.092	25.358	24	2	16	12
Zeerust	ZEE	25.129	26.158	6	3	6	5
Total				**1079**		**733**	

*Indicates that the shrew group has been included here as a host species

Table 2.2 Mean annual temperature and annual rainfall recorded at the three trapping localities that were sampled on four occasions, indicating rainfall season and vegetation type.

Locality	Mean Temperature (°C)	Annual Rainfall (mm)	Rainfall Season	Vegetation Type
GPR	16.5-28.3	106	Winter	Succulent Karoo
MBR	15.6-26.3	673	Winter	Fynbos
KPR	18.3-27.5	573	Summer	Savanna

Carcasses were either frozen in the field and remained frozen until parasite removal in the laboratory, or gastrointestinal tracts (GIT), from and including the oesophagus to the anus were removed immediately and stored in 70% alcohol for later dissection. Each individual was sexed, weighed, measured (snout-to-vent and tail length) and its reproductive state recorded. Reproductive state of females was assessed visually as either having a perforated vagina (reproductively active) or not and that of reproductive males as being visibly scrotal (reproductively active) or not (Foster 1934; Michener 1983, 1998). All helminths (total counts) were recovered from the stomach, small intestine and large intestine and stored in 70% alcohol. The number of nematodes and cestodes were logged according to Haukisalmi and Henttonen (2001). Helminth identifications were based on relevant keys and descriptions of various authors. Cestodes commonly include morphologically indistinguishable (cryptic) species (e.g. Haukisalmi et al. 2008, 2009). Therefore, nucleotide sequences of a region of nuclear DNA

coding for 28S ribosomal RNA (ca. 1400 bp) and a region of mitochondrial DNA coding for 16S-12S ribosomal RNA (ca. 810 bp) were used as additional identification tools for cestodes. The 28S region was used for Hymenolepididae (*Rodentolepis* spp.), Catenotaeniidae (*Skrjabinotaenia* spp. and *Meggittina* spp.) and Davaineidae (*Raillietina* spp. and *Inermicapsifer* spp.), and the region 16S-12S for Catenotaeniidae. The primers and amplification procedures used for 28S have been described in Littlewood et al. (2000), Lockyer et al. (2003) and Haukisalmi et al. (2016), and those for 16S-12S in Galbreath and Hoberg (2012) and von Nickisch-Rosenegk et al. (2001). The DNA sequences will be published in forthcoming molecular systematic analyses.

Voucher specimens of each species have been deposited in the National Collection of Animal Helminths at the ARC-Onderstepoort Veterinary Institute, South Africa (accession number of nematodes: S/2016/38) and the Finnish Museum of Natural History, Luomus, Finland (accession numbers of cestodes: KN 3646-KN 3668). Authorities of host and parasite species collected during the present study are listed in Tables 2.3 and 2.5, respectively.

Data analysis

For each helminth species, we calculated the host species richness (number of host species in which this parasite occurred), overall prevalence (the number of infected hosts divided by the number of hosts examined given as a percentage) and overall mean abundance (number of helminth individuals divided by total number of host individuals examined) following Bush et al. (1997). In addition, the prevalence and mean abundance of a given parasite species or taxon was calculated for each host species or taxon. Spatial distribution maps were constructed using QGIS (version 2.18.2; QGIS Development Team, 2016). Helminth species (n = 23) occurring

at three or more localities were selected to map their spatial distribution with graphic representation of abundance (Figs. 2.2, 2.3; Appendix A, S. 1).

For analysis of the temporal effect on helminth parasite parameters, we calculated the parasite species richness, the prevalence and the overall abundance per individual host of *R. pumilio* and *R. dilectus* at respective trapping sites (*R. pumilio* at GPR, n = 120 and MBR, n = 117; and *R. dilectus* at KPR, n = 97). For the abundance, helminth numbers were log transformed to approach normality. As predictor variables, we included season (winter / summer), in our generalized linear models. The data from winter and autumn were pooled as 'winter season' and that from summer and spring as 'summer season'; mid-winter considered as the middle of July and mid-summer, the middle of January. The species richness model was calculated using a Gaussian error distribution; prevalence models were calculated using a binomial error distribution and abundance using a poisson error distribution. Statistical tests were performed in R (version 3.3.3.; R Core Team, 2016), applying the MASS package (Venables and Ripley 2002). Data and plot exploration was conducted using Deducer, a data analysis GUI for R (Fellows 2012).

Results

In total, 56 helminth species were recovered from at least 16 species of rodents, shrews and sengis (n = 1079) (Tables 2.3, 2.4 and 2.5). The helminth species represented 26 genera of which 16 were nematodes (*Abbreviata* Travassos, 1920; *Ascarops* van Beneden, 1873; *Aspiculuris* Schulz, 1924; *Heligmonina* Baylis, 1928; *Mastophorus* Diesing, 1853; *Maupasina* Seurat, 1913; *Nematodirus* Ransom, 1907; *Neoheligmonella* Durette-Desset, 1971; *Paralibyostrongylus* Ortlepp, 1939; *Protospirura* Seurat, 1914; *Pseudabbreviata* Lichtenfels and Quigley, 1968; *Streptopharagus* Blanc, 1912; *Subulura* Molin, 1860; *Syphacia* Seurat, 1916; *Trichostrongylus* Loos, 1905 and *Trichuris* Roederer, 1761), nine cestodes (*Afrobaeria*

Haukisalmi, 2008; *Hymenolepis* Weinland, 1858; *Inermicapsifer* Janicki, 1910; *Mathevotaenia* Akhumyan, 1946; *Meggittina* Lynsdale, 1953; *Raillietina* Fuhrmann, 1920; *Rodentolepis* Spasskii, 1954; *Skrjabinotaenia* Akhumyan, 1946; *Sudarikovina* Spasskii, 1951) and one acanthocephalan (*Moniliformis* Travassos, 1915) (Table 2.4). The most abundant helminth species was the monoxenous nematode *Heligmonina spira* (133.8 ± 13.5) followed by *Neoheligmonella* sp. (6.9 ± 1.2) and *Syphacia* sp. (6.7 ± 1.0). *Heligmonina spira* was also the most prevalent (26.1%) followed by *Neoheligmonella capensis* (23.0%) and *Syphacia* sp. (22.2%) (Table 2.4). In general, heteroxenous nematodes and cestodes were present in lower abundance compared to monoxenous nematodes (Table 2.4). However, it is interesting to note that *Raillietina trapezoides* (5.2%, 0.9 ± 0.2) and *Meggittina baeri* (3.4%, 0.3 ± 0.1), the two species that occurred in both the western and eastern collection localities, displayed the highest prevalence and mean abundance of all cestode species.

In the present study, 11 helminth species with uncertain identity, and thus potentially new species, were recorded. These include five nematodes (one from the genus *Paralibyostrongylus*, three *Heligmonina* and one *Neoheligmonella* species) and six cestode species (two from the genus *Inermicapsifer*, and four from the genus *Skrjabinotaenia*) (Table 2.4). All of these species were recorded in rodents: four from *R. pumilio*, two from *Dasymus* sp. and one each from *Lemniscomys rosalia*, *Mastomys coucha*, *Micaelamys namaquensis*, *Mus* spp., *R. dilectus* and *Saccostomus campestris* (Table 2.5).

Table 2.3 Small mammal species trapped at various localities in South Africa, indicating food preference (FP; I = invertebrates, P = plant material, S = seeds - in order of preference), the number of individuals (N), number / (%) infected (INF), helminth species richness (SR) and number of localities where host occurred (LOC).

Host species by family	*FP	N	INF	SR	LOC
Muridae					
Aethomys chrysophilus[1] (de Winton)	S/P/I	10	10/(100)	6	2
Aethomys sp.[1]	S/P/I	2	2/(100)	2	1
Dasymys sp.[1]	P/I	4	2/(50)	3	1
Gerbilliscus brantsii[2] (Smith)	S/P/I	20	13/(65)	11	1
Lemniscomys rosalia[1] Thomas	S/P/I	17	14/(82.3)	10	3
Mastomys coucha[1] (Smith)	S/I/P	87	52/(59.8)	13	5
Mastomys natalensis[1] (Smith)	S/P/I	26	22/(84.6)	5	1
Micaelamys namaquensis[1] (A. Smith)	P/S/I	35	22/(82.9)	10	5
Mus spp.[1]	S/P/I	13	5/(38.5)	6	4
Mus musculus[1] Linnaeus	S/P/I	13	1/(7.7)	1	1
Otomys spp.[3]	P	25	13/(52)	5	5
Rhabdomys dilectus[1] (de Winton)	S/P/I	292	230/(78.8)	26	10
Rhabdomys pumilio[1] (Sparrman)	S/P/I	486	328/(67.5)	16	10
Saccostomus campestris[4] Peters	P/S/I	5	5/(100)	6	1
Steatomys pratensis[5] Peters	S/I/P	12	5/(41.7)	6	2
Soricidae					
Shrews	I	30	7/(23.3)	3	7
Macroscelididae					
Elephantulus sp.	I/P	2	2/(100)	1	1
Total		**1079**	**733/(67.9)**		

*According to Skinner and Chimimba (2005)
Rodent subfamilies according to Wilson and Reeder (2005)
[1] = Murinae; [2] = Gerbillinae; [3] = Otomyinae; [4] = Cricetomyinae; [5] = Dendromurinae

Table 2.4 Helminth species (n = 56) recorded in small mammal species in South Africa. Data presented include number of host species (HS), number of infected individuals (IH), total abundance (TA), Prevalence (%P), Mean abundance ± standard error (MA ± SE) and the number of localities positive for a given helminth species (LOC).

Helminth species	HS	IH	TA	%P	MA ± SE	LOC
Nematodes-monoxenous						
Trichostrongylus probolurus	1	46	1258	4.3	1.2 ± 0.4	4
Trichostrongylus sp.	3	32	1345	3.0	1.3 ± 0.4	6
Paralibyostrongylus sp.	2	26	185	2.4	0.2 ± 0.1	4
Paralibyostrongylus sp. 1	1	31	125	2.9	0.1 ± 0.03	1
Heligmonina boomkeri	6	87	1872	8.1	1.7 ± 0.3	5
Heligmonina spira	3	282	144376	26.1	133.8 ± 13.5	8
Heligmonina sp.	2	1	5	0.1	0.01 ± 0.004	2
Heligmonina sp. 1	1	2	41	0.2	0.04 ± 0.03	1
Heligmonina sp. 2	1	2	43	0.2	0.04 ± 0.03	1
Heligmonina sp. 3	1	2	25	0.2	0.02 ± 0.02	1
Neoheligmonella capensis	5	248	5190	23.0	4.8 ± 0.6	11
Neoheligmonella sp.	2	83	7404	7.7	6.9 ± 1.2	4
Neoheligmonella sp. 1	1	27	225	2.5	0.2 ± 0.1	1
Nematodirus sp.	2	5	106	0.5	0.1 ± 0.1	1
Syphacia obvelata	2	21	626	2.0	0.6 ± 0.2	2
Syphacia nigeriana	1	1	15	0.1	0.01 ± 0.01	1
Syphacia sp.	14	239	7240	22.2	6.7 ± 1.0	23
Aspiculuris shikoloueta	2	30	622	2.8	0.6 ± 0.2	3
Trichuris muris	5	18	101	1.7	0.1 ± 0.1	6
Trichuris sp.	1	1	1	0.1	0.001 ± 0.001	1
Nematodes-heteroxenous						
Maupasina weissi	2	4	39	0.4	0.04 ± 0.02	1
Subulura ortleppi	4	6	630	0.6	0.6 ± 0.5	2
Abbreviata sp.	3	5	20	0.5	0.02 ± 0.01	3
Pseudabbreviata sp.	1	1	9	0.1	0.01 ± 0.01	1
Protospirura muricola	4	16	113	1.5	0.1 ± 0.1	4
Protospirura numidica	5	24	287	2.2	0.3 ± 0.1	6
Protospirura sp.	4	5	6	0.5	0.01 ± 0.003	3
Ascarops strongylina	1	1	7	0.1	0.01 ± 0.01	1
Ascarops sp.	1	3	35	0.3	0.03 ± 0.03	3
Streptopharagus lerouxi	1	1	1	0.1	0.001 ± 0.001	1
Streptopharagus sp.	2	2	2	0.2	0.002 ± 0.001	1
Mastophorus muris	1	2	22	0.2	0.02 ± 0.02	2

Cestodes						
Afrobaeria acanthocirrosa	1	3	3	0.3	0.003 ± 0.002	1
Inermicapsifer sp.	5	28	66	2.6	0.1 ± 0.01	7
Inermicapsifer sp. 1	1	1	2	0.1	0.002 ± 0.002	1
Inermicapsifer sp. 2	1	1	2	0.1	0.002 ± 0.002	1
Mathevotaenia symmetrica	1	2	8	0.2	0.01 ± 0.01	1
Meggittina baeri	2	37	358	3.4	0.3 ± 0.1	4
Raillietina trapezoides	3	56	996	5.2	0.9 ± 0.2	5
Hymenolepididae	2	7	7	0.7	0.01 ± 0.002	4
Hymenolepis diminuta s.l.	1	1	1	0.1	0.001 ± 0.001	1
Rodentolepis cf. *fraterna*	1	4	5	0.4	0.01 ± 0.002	3
Rodentolepis fraterna	3	4	8	0.4	0.01 ± 0.004	3
Rodentolepis sp.	4	23	118	2.1	0.1 ± 0.04	6
Rodentolepis microstoma	7	12	45	1.1	0.04 ± 0.02	8
Rodentolepis taterae	1	4	22	0.4	0.02 ± 0.02	1
Skrjabinotaenia lobata	1	2	2	0.2	0.002 ± 0.001	1
Skrjabinotaenia lucida	2	3	8	0.3	0.01 ± 0.01	2
Skrjabinotaenia occidentalis	1	7	11	0.7	0.01 ± 0.004	1
Skrjabinotaenia sp.	1	5	13	0.5	0.01 ± 0.01	1
Skrjabinotaenia sp. 1	1	5	185	0.5	0.2 ± 0.1	1
Skrjabinotaenia sp. 2	1	8	26	0.7	0.02 ± 0.01	2
Skrjabinotaenia sp. 3	2	8	34	0.7	0.03 ±0.01	2
Skrjabinotaenia sp. 4	1	1	1	0.1	0.001 ± 0.001	1
Sudarikovina sp.	2	4	24	0.4	0.02 ± 0.01	2
Acanthocephala						
Moniliformis sp.	1	1	27	0.09	0.03 ± 0.03	1

Table 2.5 Prevalence (%P), mean abundance (MA ± SE) of individual nematode and cestode species recovered from rodent host species (n = number of host individuals) in South Africa, also indicating previous records.

		Aethomys chrysophilus (n = 10)	*Aethomys* sp. (n = 2)	*Dasymys* sp. (n = 4)	*Gerbilliscus brantsii* (n = 20)	*Lemniscomys rosalia* (n = 17)	*Mastomys coucha* (n = 87)	*Mastomys natalensis* (n = 26)	*Micaelamys namaquensis* (n = 35)	*Mus* spp. (n = 13)	*Mus musculus* (n = 13)	*Otomys* sp. (n = 25)	*Rhabdomys dilectus* (n = 292)	*Rhabdomys pumilio* (n = 486)	*Saccostomus campestris* (n = 5)	*Steatomys pratensis* (n = 12)
Nematodes-monoxenous																
Trichostrongylus probolurus (Railliet, 1896)	%P	—	—	—	—	—	—	—	—	—	—	—	—	9.5[c]	—	—
	MA±SE													2.6±0.9		
Trichostrongylus sp.	%P	—	—	—	5.0**	—	—	—	—	—	—	—	10.3**	0.2	—	—
	MA±SE				0.2±0.2								4.6±1.3	0.02±0.02		
Paralibyostrongylus sp.	%P	—	—	—	—	—	—	—	—	—	—	1.0	6.9**	—	—	—
	MA±SE											4.4±1.3	0.4±0.1			
Paralibyostrongylus sp. 1	%P	—	—	—	—	—	—	—	—	—	—	—	—	6.4**	—	—
	MA±SE													0.3±0.1		
Heligmonina boomkeri Durette-Desset and Diginani, 2005	%P	70.0[b]	—	—	—	41.2*	33.3*	69.2*	—	—	—	—	8.6*	—	—	8.3*
	MA±SE	20.1±7.1				4.4±1.6	7.1±1.9	34.0±8.8					0.3±0.1			0.2±0.2
Heligmonina spira (Ortlepp, 1939)	%P	20.0*	—	—	—	—	—	—	—	—	—	—	18.8*	46.3[b,c,e,]	—	—
	MA±SE	0.4±0.3											19.4±4.2	29.4±28.5		
Heligmonina sp.	%P	—	—	—	—	—	—	—	—	—	—	4.0**	0.3	—	—	—
	MA±SE											0.2±0.2	0.003±0.003			
Heligmonina sp. 1	%P	—	—	50.0**	—	—	—	—	—	—	—	—	—	—	—	—
	MA±SE			10.3±6.3												
Heligmonina sp. 2	%P	—	—	50.0**	—	—	—	—	—	—	—	—	—	—	—	—
	MA±SE			10.8±7.0												

		Aethomys chrysophilus (n = 10)	*Aethomys* sp. (n = 2)	*Dasymys* sp. (n = 4)	*Gerbilliscus brantsii* (n = 20)	*Lemniscomys rosalia* (n = 17)	*Mastomys coucha* (n = 87)	*Mastomys natalensis* (n = 26)	*Micaelamys namaquensis* (n = 35)	*Mus* spp. (n = 13)	*Mus musculus* (n = 13)	*Otomys* sp. (n = 25)	*Rhabdomys dilectus* (n = 292)	*Rhabdomys pumilio* (n = 486)	*Saccostomus campestris* (n = 5)	*Steatomys pratensis* (n = 12)
Heligmonina sp. 3	%P	—	—	—	—	—	—	—	—	—	—	—	—	0.4**	—	—
	MA±SE													0.1±0.1		
Neoheligmonella capensis (Ortlepp, 1939)	%P	—	—	—	—	11.8*	0.03*	—	—	—	—	0.2	37.3*	28.0[b,c]	—	—
	MA±SE					0.5±0.4	0.03±0.03					0.7±0.4	8.6±1.7	5.5±0.6		
Neoheligmonella sp.	%P	—	—	—	—	—	—	—	—	—	—	1.0	26.4	—	—	—
	MA±SE											14.8±4.2	24.5±4.3			
Neoheligmonella sp. 2	%P	—	—	—	—	—	—	—	—	—	—	—	—	5.6**	—	—
	MA±SE													0.5±0.2		
Nematodirus sp.	%P	—	—	—	—	—	—	—	2.9**	—	—	—	—	0.8**	—	—
	MA±SE								1.5±2.6					0.1±0.1		
Syphacia obvelata (Rudolphi, 1802)	%P	—	—	—	—	—	23.0*	—	—	—	—	—	0.3*	—	—	—
	MA±SE						7.2±0.7						0.003±0.003			
Syphacia nigeriana Baylis, 1928	%P	—	—	—	5.0*	—	—	—	—	—	—	—	—	—	—	—
	MA±SE				0.8±0.8											
Syphacia sp.	%P	—	6.5**	50.0**	30.0	11.8**	11.5	19.2**	34.3**	15.4**	7.7	—	30.1	21.4	60.0**	16.7**
	MA±SE		6.5±5.0	10.8±6.9	21.1±44.8	1.6±1.5	2.2±1.2	4.6±3.7	6.3±4.8	1±0.7	2.4±2.4		10.2±2.2	6.4±1.6	4.0±1.9	5.5±3.7
Aspiculuris shikoloueta Inglis, Harris and Lewis, 1990	%P	—	—	—	—	—	—	—	2.9[d]	—	—	—	—	6.0*	—	—
	MA±SE								0.2±0.3					1.3±0.4		
Trichuris muris (Schrank, 1788)	%P	—	—	—	—	—	6.9*	15.4[h]	—	—	—	—	1.03*	0.4[c]	60.0*	—
	MA±SE						0.8±0.7	0.5±0.3					0.01±0.01	0.004±0.003	2.4±1.7	
Trichuris sp.	%P	—	—	—	—	—	—	—	—	—	—	—	0.3	—	—	—
	MA±SE												0.003±0.003			

		Aethomys chrysophilus (n = 10)	*Aethomys* sp. (n = 2)	*Dasymys* sp. (n = 4)	*Gerbilliscus brantsii* (n = 20)	*Lemniscomys rosalia* (n = 17)	*Mastomys coucha* (n = 87)	*Mastomys natalensis* (n = 26)	*Micaelamys namaquensis* (n = 35)	*Mus* spp. (n = 13)	*Mus musculus* (n = 13)	*Otomys* sp. (n = 25)	*Rhabdomys dilectus* (n = 292)	*Rhabdomys pumilio* (n = 486)	*Saccostomus campestris* (n = 5)	*Steatomys pratensis* (n = 12)
Nematodes-heteroxenous																
Maupasina weissi Seurat, 1913	%P	—	—	—	—	—	—	—	5.7*	—	—	—	—	—	—	—
	MA±SE								0.1±0.2							
Subulura ortleppi Inglis, 1960	%P	20.0*	—	—	—	5.9*	2.3*	—	—	7.7*	—	—	—	—	—	—
	MA±SE	58.3±52.6				0.5±0.5	0.4±0.3			0.6±0.6						
Abbreviata sp.	%P	—	—	—	—	5.9**	—	—	5.7**	—	—	—	0.7**	—	—	—
	MA±SE					0.1±0.1			0.1±0.11				0.1±0.1			
Protospirura muricola Gedoelst, 1916	%P	—	—	—	20.0*	—	1.6*	—	8.6*	—	—	—	2.7*	—	—	—
	MA±SE				2.5±2.2		0.02±0.02		0.3±0.4				0.2±0.1			
Protospirura numidica Seurat, 1914	%P	20.0*	—	—	—	5.9*	12.6*	—	—	—	—	—	2.7*	—	—	16.7*
	MA±SE	2.2±1.7				2.6±2.6	0.9±0.4						0.4±0.4			1.5±1.2
Protospirura sp.	%P	—	—	—	5.0	—	1.2	—	—	7.7**	—	—	0.7	—	—	—
	MA±SE				0.1±0.1		0.01±0.11			0.1±0.1			0.01±0.01			
Ascarops strongylina (Rudolphi, 1819)	%P	—	—	—	—	—	—	—	—	—	—	—	0.3*	—	—	—
	MA±SE												0.02±0.02			
Ascarops sp.	%P	—	—	—	—	—	—	—	—	—	—	—	1.0	—	—	—
	MA±SE												0.1±11			
Streptopharagus lerouxi Quentin, 1964	%P	—	—	—	—	—	—	—	—	—	—	—	0.3*	—	—	—
	MA±SE												0.003±0.003			
Streptopharagus sp.	%P	—	—	—	5.0**	—	—	—	—	7.7**	—	—	—	—	—	—
	MA±SE				0.1±0.1					0.1±0.1						
Mastophorus muris (Gmelin, 1790)	%P	—	—	—	—	—	—	—	—	—	—	—	0.7*	—	—	—
	MA±SE												0.1±0.1			

		Aethomys chrysophilus (n = 10)	*Aethomys* sp. (n = 2)	*Dasymys* sp. (n = 4)	*Gerbilliscus brantsii* (n = 20)	*Lemniscomys rosalia* (n = 17)	*Mastomys coucha* (n = 87)	*Mastomys natalensis* (n = 26)	*Micaelamys namaquensis* (n = 35)	*Mus* spp. (n = 13)	*Mus musculus* (n = 13)	*Otomys* sp. (n = 25)	*Rhabdomys dilectus* (n = 292)	*Rhabdomys pumilio* (n = 486)	*Saccostomus campestris* (n = 5)	*Steatomys pratensis* (n = 12)
Cestodes																
Afrobaeria acanthocirrosa (Baer, 1924)	%P	—	—	—	—	—	—	—	—	—	—	0.5*	—	—	—	—
	MA±SE											0.2±0.1				
Inermicapsifer sp.	%P	50.0	—	—	—	11.8**	2.3**	7.7**	—	—	—	—	5.8**	—	—	—
	MA±SE	1.5±0.5				0.6±0.4	0.03±0.03	0.1±0.1					0.1±0.04			
Inermicapsifer sp. 1	%P	—	—	—	—	—	1.2	—	—	—	—	—	—	—	—	—
	MA±SE						0.02±0.02									
Inermicapsifer sp. 2	%P	—	—	—	—	5.9	—	—	—	—	—	—	—	—	—	—
	MA±SE					0.1±0.01										
Mathevotaenia symmetrica (Baylis, 1927)	%P	—	—	—	—	—	—	—	5.7*	—	—	—	—	—	—	—
	MA±SE								0.2±0.3							
Meggittina baeri Lynsdale, 1953	%P	—	—	—	—	—	—	—	—	—	—	—	5.1*	4.5*	—	—
	MA±SE												0.6±0.2	0.4±0.1		
Raillietina trapezoides (Janicki, 1904)	%P	—	—	—	—	—	—	—	5.7*	—	—	—	5.8*	7.6[f]	—	—
	MA±SE								0.1±0.11				0.7±0.2	1.7±0.4		
Hymenolepididae	%P	—	—	—	—	5.9	—	—	—	—	—	—	—	—	—	—
	MA±SE					0.1±0.1										
H. diminuta s.l.	%P	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8.3**
	MA±SE															0.1±0.1
Rodentolepis cf. *fraterna* (Stiles, 1906)	%P	—	—	—	—	—	—	—	—	—	—	—	1.37	—	—	—
	MA±SE												0.02±0.01			
Rodentolepis fraterna (Stiles, 1906)	%P	—	1.5*	—	10.0*	—	—	—	—	—	—	—	—	—	20.0*	—
	MA±SE		1.5±50		0.2±0.1										0.2±0.2	

		Aethomys chrysophilus (n = 10)	*Aethomys* sp. (n = 2)	*Dasymys* sp. (n = 4)	*Gerbilliscus brantsii* (n = 20)	*Lemniscomys rosalia* (n = 17)	*Mastomys coucha* (n = 87)	*Mastomys natalensis* (n = 26)	*Micaelamys namaquensis* (n = 35)	*Mus* spp. (n = 13)	*Mus musculus* (n = 13)	*Otomys* sp. (n = 25)	*Rhabdomys dilectus* (n = 292)	*Rhabdomys pumilio* (n = 486)	*Saccostomus campestris* (n = 5)	*Steatomys pratensis* (n = 12)
Rodentolepis sp.	%P	—	—	—	25.0	—	2.3	—	—	—	—	—	4.8	—	40.0	—
	MA±SE				2.1±1.3		0.02±0.02						0.2±0.1		2.6±1.7	
Rodentolepis microstoma (Dujardin, 1845)	%P	—	—	—	5.0*	5.9*	2.3*	—	—	7.7*	—	—	1.7*	—	20.0*	8.3*
	MA±SE				0.1±0.1	0.1±0.1	0.1±0.03			0.4±0.40			0.1±0.1		0.2±0.2	0.1±0.1
Rodentolepis taterae Collins, 1972	%P	—	—	—	20.0[a]	—	—	—	—	—	—	—	—	—	—	—
	MA±SE				1.1±0.9											
Skrjabinotaenia lobata (Baer, 1925)	%P	—	—	—	—	—	—	7.7*	—	—	—	—	—	—	—	—
	MA±SE							0.1±0.1								
Skrjabinotaenia lucida (Ortlepp, 1962)	%P	20.0[g]	—	—	—	—	—	—	2.9*	—	—	—	—	—	—	—
	MA±SE	0.3±0.2							0.1±0.2							
Skrjabinotaenia occidentalis Hunkeler, 1972	%P	—	—	—	—	—	—	—	—	—	—	—	—	1.4*	—	—
	MA±SE													0.02±0.01		
Skrjabinotaenia sp.	%P	—	—	—	—	—	—	—	—	—	—	—	—	1.0	—	—
	MA±SE													0.03±0.01		
Skrjabinotaenia sp. 1	%P	—	—	—	—	—	—	—	—	—	—	—	—	—	100.0**	—
	MA±SE														37.0±26.6	
Skrjabinotaenia sp. 2	%P	—	—	—	—	—	—	—	22.9**	—	—	—	—	—	—	—
	MA±SE								0.7±0.5							
Skrjabinotaenia sp. 3	%P	—	—	—	—	—	—	—	—	—	—	—	1.4**	0.8	—	—
	MA±SE												0.04±0.02	0.1±0.03		
Skrjabinotaenia sp. 4	%P	—	—	—	—	—	—	—	—	7.7**	—	—	—	—	—	—
	MA±SE									0.1±0.10						

		Aethomys chrysophilus (n = 10)	*Aethomys* sp. (n = 2)	*Dasymys* sp. (n = 4)	*Gerbilliscus brantsii* (n = 20)	*Lemniscomys rosalia* (n = 17)	*Mastomys coucha* (n = 87)	*Mastomys natalensis* (n = 26)	*Micaelamys namaquensis* (n = 35)	*Mus* spp. (n = 13)	*Mus musculus* (n = 13)	*Otomys* sp. (n = 25)	*Rhabdomys dilectus* (n = 292)	*Rhabdomys pumilio* (n = 486)	*Saccostomus campestris* (n = 5)	*Steatomys pratensis* (n = 12)
Sudarikovina sp.	%P	—	—	—	15.0**	—	—	—	—	—	—	—	—	—	—	8.3**
	MA±SE				1.0±0.7											0.4±0.4

*First time this helminth species is recorded from this host species or genus
**First time this helminth genus is recorded from this host species or genus
References (previous records): [a]Collins, 1972; [b]Durette-Desset and Digiani, 2005; [c]Froeschke et al. 2010; [d]Inglis et al. 1990; [e]Ortlepp, 1939; [f]Ortlepp, 1940; [g]Ortlepp, 1962; [h]Verster, 1960.

Host association of helminth species

Most of the helminth species (n = 27; 47%) were recovered from a single host species (Table 2.4 and 2.5). However, several helminth species (n = 11) were recorded from four or more host species and even across host families. The nematode *Syphacia* sp. was recorded in 13 rodent species and shrews. The cestode *Rodentolepis microstoma* was recorded in seven murids, representing four subfamilies [*Gerbilliscus brantsii* (Gerbillinae), *L. rosalia*, *M. coucha*, *Mus* spp., *R. dilectus* (Murinae), *S. campestris* (Cricetomyinae) and *Steatomys pratensis* (Dendromurinae); see Table 2.3], of which the highest prevalence was recorded in *S. campestris* (20%). *Heligmonina boomkeri* was recorded in five murine species (*Aethomys chrysophilus, L. rosalia, M. coucha, Mastomys natalensis, R. dilectus*) as well as in *S. pratensis.* It reached the highest prevalence in *A. chrysophilus* and *M. natalensis* (70% and 69.2%, respectively), the latter having a marginally higher abundance than the former (34.0 ± 8.8 and 20.1 ± 7.1, respectively) (Table 2.5). Three nematodes (*N. capensis*, *Protospirura numidica* and *Trichuris muris*) and a cestode (*Inermicapsifer* sp.) recorded a host range of five rodent species (Table 2.5). Each of these species showed some level of host preference with *N. capensis* more commonly associated with *R. dilectus*, *T. muris* with *S. campestris* and *P. numidica* and *Inermicapsifer* sp. with *A. chrysophilus* (Table 2.5). In general, heteroxenous nematodes and cestodes were present in fewer host species and at a lower level of infection compared to monoxenous nematodes (Table 2.4). The only acanthocephalan, *Moniliformis* sp., was recovered from *M. coucha* (0.1%; 0.03 ± 0.03), which represents a new genus record from this host (Table 2.4).

Of the helminths identified to species level (n = 25), one or several new host records were established for 14 nematodes and nine cestodes (Table 2.5). The nematode with the largest number of new host records was *H. boomkeri* (five), while seven new host records were

established for the cestode *R. microstoma*. In addition, nine nematode and four cestode genera could for the first time be associated with one or several given host species or genera (Table 2.5).

The number of helminth species varied between small mammal host species. The two most abundant host species *R. pumilio* (n = 486) and *R. dilectus* (n = 292) harboured the largest number of helminth species. *Rhabdomys dilectus* harboured 26 helminth species, 19 nematodes and seven cestodes. Of the nematodes recovered, 10 represent new host records for a parasite species and three for a genus. Of the cestodes recovered, three represent new host records for species and two for genera (Table 2.5). *Rhabdomys pumilio* harboured 16 helminth species, 11 nematodes and five cestodes, of which one and two, respectively, are new host species records. Three nematode records represent new parasite genera for this host. *Mastomys coucha* (n = 87) collected from five localities had the third largest number of helminth species (13), comprising nine nematode and four cestode species, of which seven and one, respectively, were new host species records and one a new genus record for cestodes. The acanthocephalan, *Moniliformis* sp., is also a new genus record from this host. *Micaelamys namaquensis* (n = 35) from five localities harboured 10 helminth species and *L. rosalia* (n = 17) from three localities, harboured nine helminth species as well as specimens of the family Hymenolepididae (Table 2.5). Of note is that *G. brantsii* (n = 20), collected from only one locality (WSR), harboured 11 helminth species comprising six nematode and five cestode species of which two and two, respectively, were new host species records (Table 2.5).

Shrews collected from seven localities harboured *Syphacia* sp. (monoxenous nematode) and *Pseudabbreviata* sp. (heteroxenous nematode) in equal prevalence though the former was marginally more abundant (Table 2.6). The two sengi individuals (*Elephantulus* sp.) recovered

from one locality harboured only *Maupasina weissi* (Table 2.6). This nematode was also recovered from *M. namaquensis* for the first time (Table 2.5), and at the same locality (BER), but in much lower abundance and prevalence compared to the sengis (Table 2.6).

Table 2.6 Prevalence (%P), Mean abundance (MA ± SE) of individual nematode and cestode species recovered from shrews and sengis (n = number of host individuals).

Helminth species/taxon		*Elephantulus* sp. (n = 2)	Shrews (n = 30)
Nematodes-monoxenous			
Syphacia sp.	%P	—	3.3
	MA ± SE		0.6 ± 0.6
Nematodes-heteroxenous			
Maupasina weissi	%P	100	—
	MA ± SE	17.0 ± 3.0	
Pseudabbreviata sp.	%P	—	3.3
	MA ± SE		0.3 ± 0.3
Cestodes			
Hymenolepididae	%P	—	0.2
	MA ± SE		0.2 ± 0.1

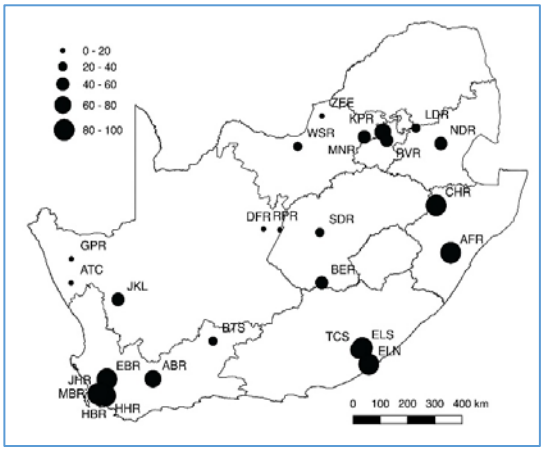
Geographic distribution of helminth species

Maps depicting the spatial distribution of the individual helminth species that were recorded at three or more localities (n = 23) are provided in Appendix A (S. 1). The 15 nematode (10 monoxenous and five heteroxenous), and eight cestode species varied in geographic extent. However, some patterns were evident: monoxenous nematode species were recovered from across South Africa (Fig. 2.2A), while heteroxenous nematodes occurred only in the eastern region of South Africa (Fig. 2.2B). Although cestodes showed a countrywide distribution in the combined map (Fig. 2.3A), there was clear separation with some species (e.g. *R.*

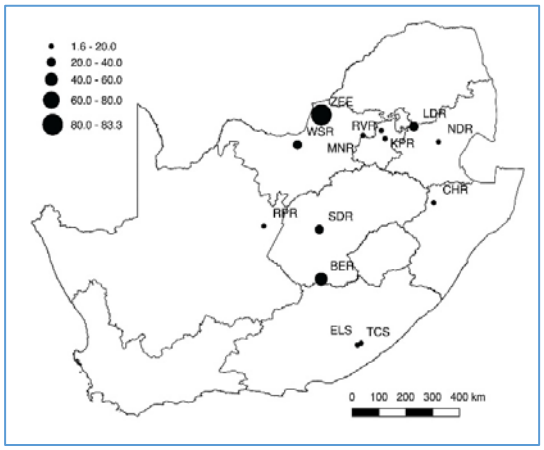
microstoma) associated with the eastern and central regions and others (e.g. *R. trapezoides*) with the western and central regions of the country.

Of the monoxenous nematode species recovered, *Syphacia* sp. was the most widely distributed (at 23 of the 26 localities) across the country (Fig. 2.2C), followed by *N. capensis* at 11 localities (Fig. 2.2D) and *H. spira* at eight (Appendix A, S. 1). Of the heteroxenous nematodes, *P. numidica* and *Protospirura muricola* were the most widely distributed at six and four localities, respectively. Cestode species were recovered from 20 of the 26 localities (Fig. 2.3A). *Rodentolepis microstoma* was the most widely distributed cestode and was recorded at eight localities predominantly in the eastern region (Fig. 2.3B). *Inermicapsifer* sp. was the second most widely distributed cestode and occurred at seven localities (Appendix A, S. 1). The only acanthocephalan, *Moniliformis* sp. was recovered from one locality (ZEE) in the north-eastern region of South Africa (Fig. 2.1).

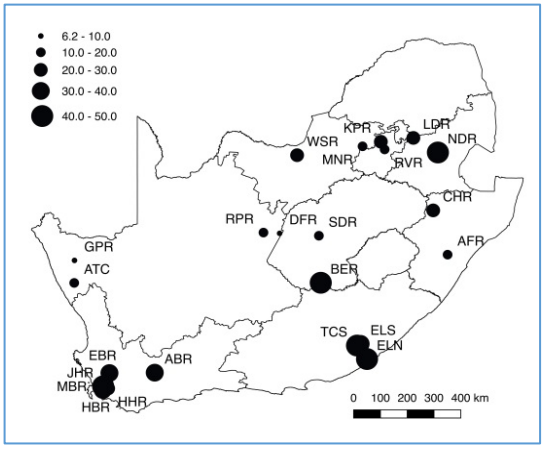
A. Nematodes Monoxenous (NM) combined



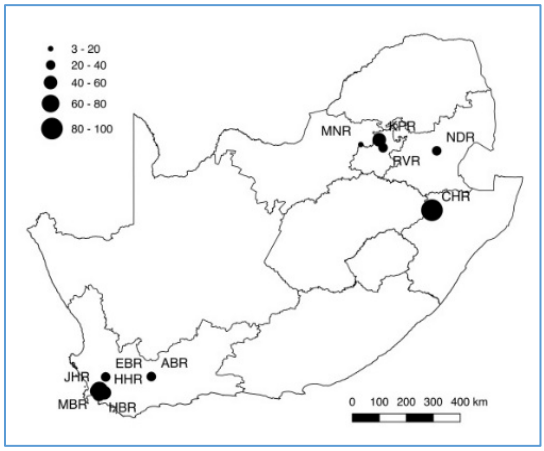
B. Nematodes Heteroxenous (NH) combined



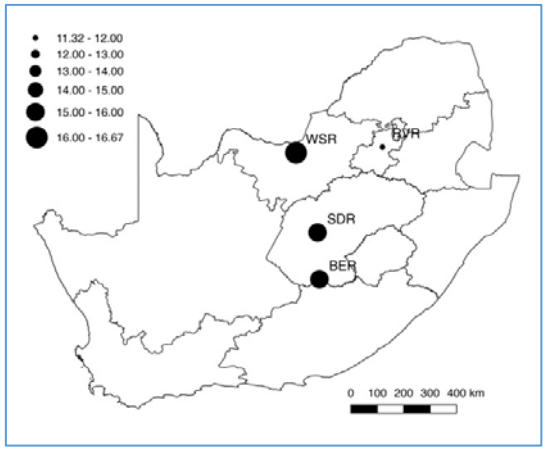
C. *Syphacia* sp. (NM)



D. *Neoheligmonella capensis* (NM)



E. *Protospirura muricola* (NH)



F. *Protospirura numidica* (NH)

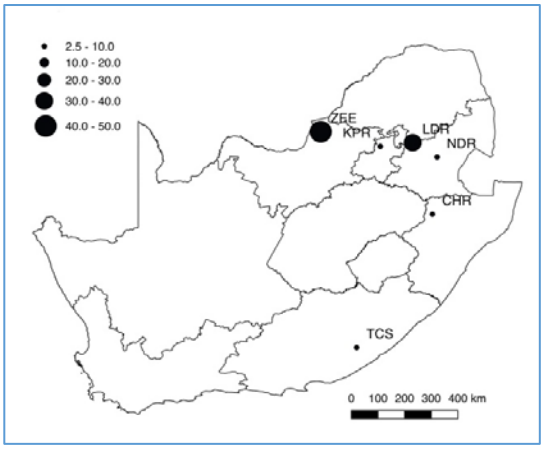
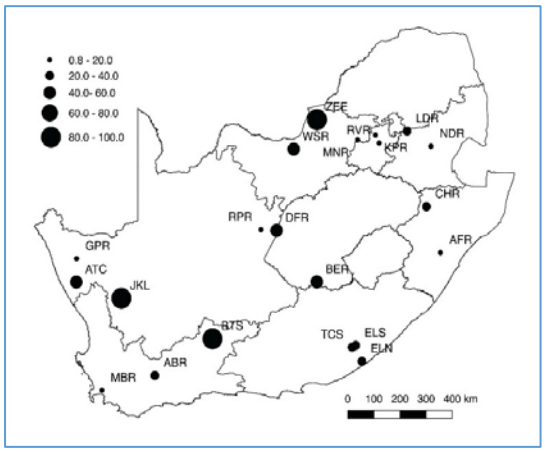
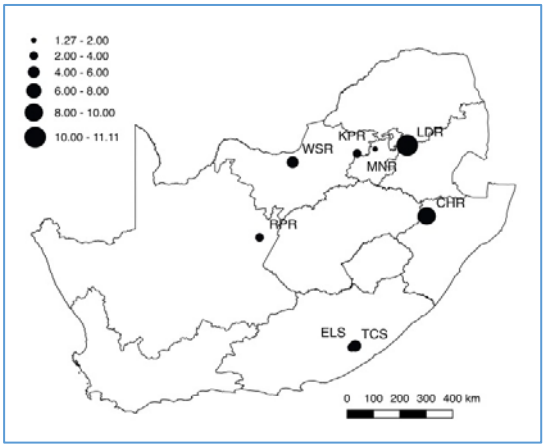


Fig. 2.2 Distribution and graphic indication of relative abundance of nematode species recorded in small mammals across South Africa. NM = nematodes with a direct life cycle; NH = nematodes with an indirect life cycle.

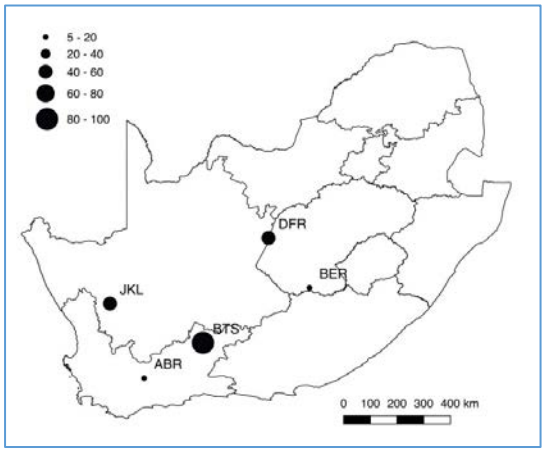
A. Cestodes combined



B. *Rodentolepis microstoma*



C. *Raillietina trapezoides*



D. *Meggittina baeri*

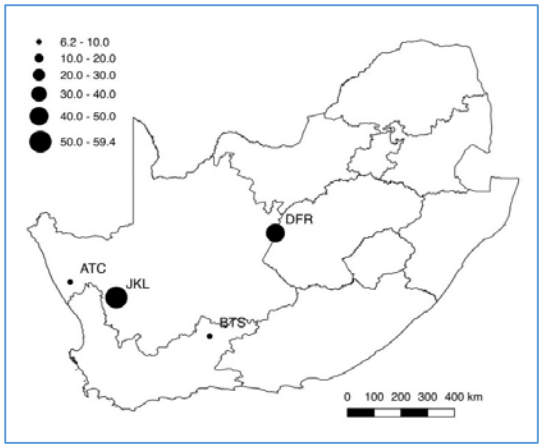
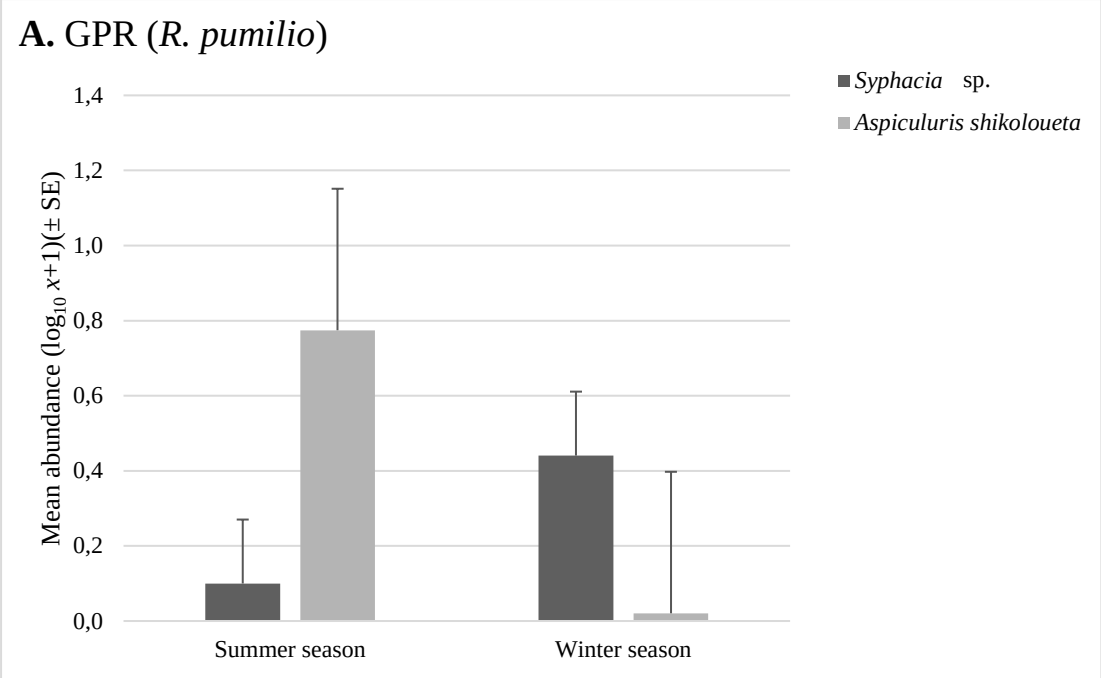
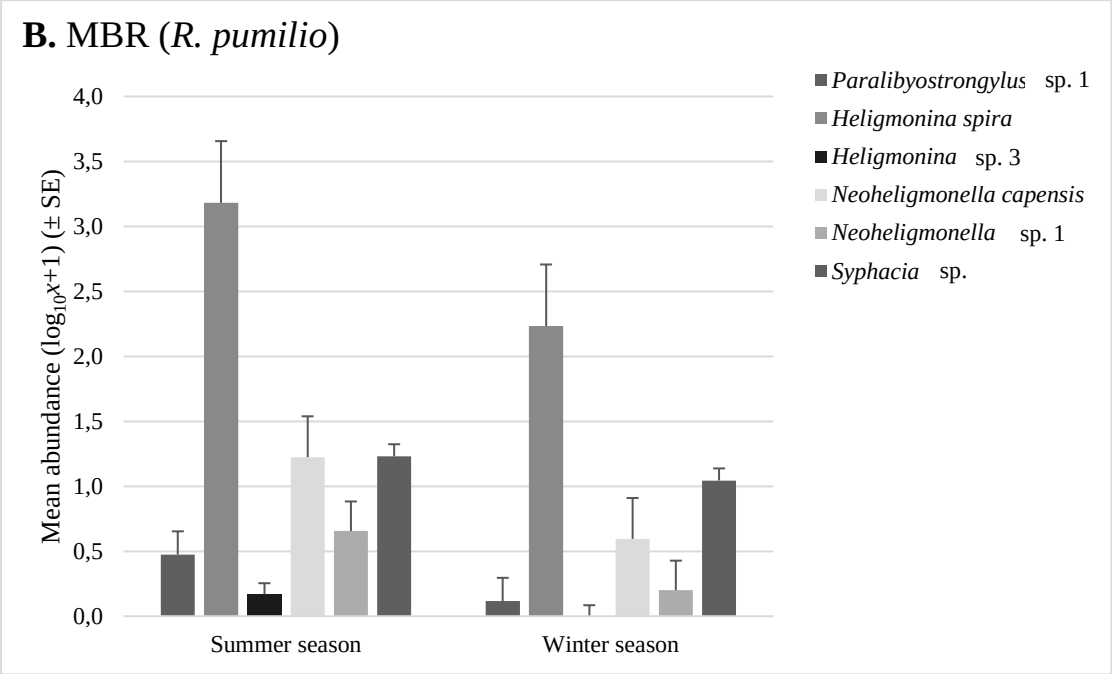


Fig. 2.3 Distribution and graphic indication of relative abundance of cestode species recorded in small mammals across South Africa.

Spatial and temporal variation in helminth abundance

The mean abundance of nematode and cestode species varied between localities and seasons. At Springbok (GPR), only two nematode species were recorded in *R. pumilio*. *Aspiculuris shikoloueta* was the most abundant in summer compared to winter, while *Syphacia* sp. displayed an opposite pattern (Fig. 2.4A). The overall mean species richness ($t = -2.309$; $P = 0.023$), mean abundance ($t = -3.604$; $P < 0.001$) and prevalence ($z = -2.144$; $P = 0.032$) of nematode species were significantly higher in summer compared to winter in *R. pumilio* at GPR. At Stellenbosch site 3 (MBR), six nematode species were recorded. All the nematode species displayed higher mean abundance in summer compared to winter (Fig. 2.4B). The overall mean abundance ($t = -2.245$; $P = 0.025$) and prevalence ($z = -2.836$; $P = 0.005$), but not mean species richness ($t = -1.083$; $P = 0.281$), of nematode species were significantly higher in summer compared to winter in *R. pumilio* at MBR. The pattern for the overall helminth infection at Pretoria site 1 (KPR) is distinctly different from the two previous localities (Fig. 2.4C). Firstly, five nematode and one cestode species were recorded in *R. dilectus* and secondly, the mean species richness ($t = 2.842$; $P = 0.006$), mean abundance ($t = 2.791$; $P = 0.005$) and prevalence ($z = 1.992$; $P = 0.046$) of all helminth species combined were significantly higher in winter compared to summer in *R. dilectus*. This pattern was supported by the two most abundant nematode species (*N. capensis* and *Syphacia* sp.), but not by the remaining four species (Fig. 2.4C).





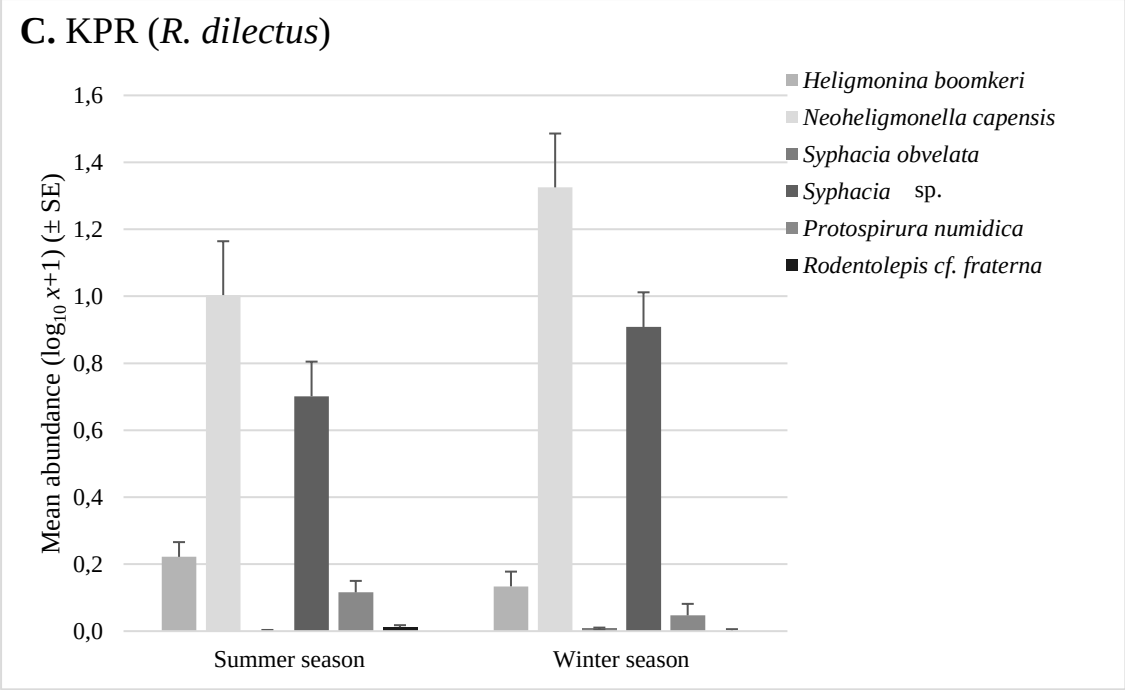


Fig. 2.4 Mean abundance ($\log_{10} x + 1$) of helminths recovered from *Rhabdomys pumilio* and *R. dilectus* during the summer and winter seasons at GPR (A), MBR (B) and KPR (C). See Table 2.1 for locality abbreviation codes.

Discussion

The study highlights the diversity and distribution of helminth species that are associated with small mammals and in particularly rodents in South Africa. More than 50 helminth species were recorded, of which most were nematodes and in particular monoxenous species followed by heteroxenous nematodes and cestodes. This pattern is similar to previous studies on rodents conducted in Britain and Spain (Lewis 1987; Fuentes et al. 2004). Most of the small mammals that were trapped are omnivores that primarily consume plant material (Skinner and Chimimba 2005) and as such, it is to be expected that they will have a higher chance of being infected by free-living infective stages that are associated with vegetation (Sousa and Grosholz 1991; Anderson 2000). Variation in the density, and thus availability, of infective stages may also contribute to this pattern. The infective stages of monoxenous taxa are normally more localised in the environment due to the fact that multiple eggs are voided with the faeces (Mas-Coma et al. 2008). This is in contrast with heteroxenous taxa where the infective stages for the definitive host would be more widely dispersed by intermediate hosts (Georgiev et al. 2006; Kinsella 1991; Mas-Coma et al. 2008).

Host association of helminth species

A large number (n = 27) of helminths displayed single host associations. However, 10 of these are likely new species and therefore have only their respective hosts as records. Literature records document a wide range of alternate hosts, especially rodents (Baylis 1928; Quentin 1964; Lichtenfels and Quigley 1968; Anderson 2000), for 14 further helminth species that displayed single host associations in this study. For example, *Ascarops strongylina*, *Streptopharagus lerouxi* and *Mastophorus muris* were all recorded from only *R. dilectus*, and not from other co-occurring rodent species. *Ascarops strongylina* (a heteroxenous nematode) has previously been recorded in several rodent taxa that are not native to South and southern

Africa. For example, the fat dormouse *Glis glis* (Linnaeus) from Europe and the Indian palm squirrel *Funambulus palmarum* (Linnaeus) from India and Sri Lanka have been reported as paratenic hosts (Ganzorig et al. 1999). Its presence in *R. dilectus* (omnivore) supports the latter authors' opinion that *A. strongylina* may have a wider definitive and paratenic host range than previously thought. *Streptopharagus lerouxi* was described from *Tatera lobengulae* (de Winton) in the Congo (Quentin 1964). In this study, it was absent from several rodent species (*M. namaquensis*, *Mus musculus*, *Otomys* sp. and *S. campestris*) that co-occurred with *R. dilectus* at the same localities. Important to note is that the mean abundance of *S. lerouxi* was very low in *R. dilectus* (0.003 ± 0.003), possibly due to a low abundance of infected intermediate hosts. The latter might also explain the absence of *M. muris*, a cosmopolitan species that has previously been recorded from rodents in Africa (Behnke et al. 2000), from all hosts but *R. dilectus*.

Based on previous studies, only two of the present helminth species, *Afrobaeria acanthocirrosa* (see Haukisalmi 2008) and *Rodentolepis taterae* (see Prakash and Ghosh 2012), displayed a likely genus-specific host association in *Otomys* sp. and gerbils (defined as *Tatera* species), respectively. These authors did not speculate on reasons for this genus specificity which most probably relates to host diet, promoting the likelihood of infected intermediate host ingestion. A similarly strong host-parasite relationship seems to connect *M. weissi* (heteroxenous nematode) and the Macroscelidea (see Lutermann et al. 2015). In the latter study, *M. weissi* was the most abundant and prevalent helminth species in the sengi *Elephantulus myurus* Thomas and Schwann, whose diet predominantly consists of invertebrates (Skinner and Chimimba 2005). The present study supports this relationship in that *M. weissi* was present in relatively large numbers (17.0 ± 3.0) in the two *Elephantulus* sp. individuals examined here.

Though *M. weissi* was also recorded in the co-occurring omnivorous rodent *M. namaquensis*, it was present in lower abundance (0.14 ± 0.20).

Several helminth species seemed to have broader host ranges and were recorded across host genera, subfamilies and even families. Even though this was the case, some helminth species did display some level of host preference. For example, *H. boomkeri* was present in six host species but reached noticeable higher abundances and prevalence in *A. chrysophilus* (20.1 ± 7.1, 70.0%) and *M. natalensis* (34.0 ± 8.8, 69.2%). In addition, the present study supports Durette-Desset and Digiani (2005) in that *H. boomkeri* is associated with *A. chrysophilus* and *N. capensis* with *R. pumilio*. The presence of *Inermicapsifer madagascariensis, P. numidica* and *Subulura ortleppi* in *A. chrysophilis* is in agreement with de Graaff (1981). The fact that *Syphacia* sp. was recorded in 13 host species, including the shrews, and at 23 of the 26 localities suggests a very broad host and geographic range. It is likely though that this record represents multiple *Syphacia* species. In the absence of male specimens, as experienced in this study, identification to species level is virtually impossible, and can only be confirmed using molecular techniques.

Medically important helminths that were recovered include the acanthocephalan, *Moniliformis* sp. from one locality (ZEE) in *M. coucha* and the cestodes *Hymenolepis diminuta* s. l. from *S. pratensis* and *R. microstoma* in a range of host species. In all cases, these helminths are known to have zoonotic potential although human infection is considered rare (Ikeh et al. 1992; Macnish et al. 2003; Salehabadi et al. 2008; Waugh et al. 2006). Species of the anoplocephalid cestode genus *Inermicapsifer* are very common in Africa, mainly in rodents and hyrax (Fourie et al. 1987; see Macpherson and Craig 1991) and in the current study representatives of this genus were collected from a number of host taxa. Within the genus *Inermicapsifer*, *I.*

madagascariensis is widespread in animals, and has been reported in humans from a number of African countries, including Kenya, South Africa, Zambia and Zimbabwe (see Macpherson and Craig 1991).

Helminth species richness varied between small mammal host species. The larger number of helminth species recorded in *R. dilectus* (n = 292) and *R. pumilio* (n = 486) may be biased by larger sample sizes compared to other more abundant rodent taxa (sample sizes ranged between 17 and 87). It is thus difficult to infer if the larger helminth species richness (26 and 16 species, respectively) recorded in the two *Rhabdomys* species is due to their large geographic range (Feliú et al. 1997; Krasnov et al. 2004b). As mentioned above, food preference influences the exposure of hosts to helminth parasites. In general, rodents feed on plant material, seeds and to a lesser extent on invertebrates (Perrin 1981; Skinner and Chimimba 2005; Kingdon et al. 2013). This largely explains the diversity of helminth types (monoxenous and heteroxenous) recorded in rodents in the present study. On the other hand, shrews are regarded as opportunistic carnivores, living entirely on a range of invertebrates that include mainly insects, but also snails and slugs (Langer 2002; Skinner and Chimimba 2005). The diet of sengis mainly comprises of invertebrates (90%) of which the majority are isopterans and formicids, followed by coleopterans and other arthropods (Skinner and Chimimba 2005). Small mammals that feed mainly on arthropods and other invertebrates are therefore expected to harbour helminth species that use invertebrates as intermediate hosts. This is supported in the present study and by Lutermann et al. (2015), who mainly recorded heteroxenous helminths from *E. myurus*. In addition, the fact that most rodents often occur in higher densities within the landscape (Arneberg et al. 1998a; Stanko et al. 2002) predisposes them to a higher rate of contact with directly transmitted helminth parasites (Arneberg et al. 1998a; Stanko et al. 2002). This is in

contrast to most shrew and sengi species that generally occur in lower abundances (Gliwicz and Taylor 2002; Ribble and Perrin 2005).

The broad host range and geographic extent of the study provides an opportunity to identify novel parasite-host associations. For example, five new host records were recorded for the monoxenous nematode *H. boomkeri*, while seven new host records were established for the cestode *R. microstoma.* This, together with the fact that 11 potentially new helminth species were recorded highlights the need for future descriptive research on helminths of small mammals and wildlife in general for South and southern Africa.

Spatial and temporal distribution of helminths shaped by the environment

In the present study, the geographic distribution of helminth species seems to be life cycle-specific with monoxenous nematodes recorded across the country, whereas heteroxenous nematodes and some cestodes were restricted to the eastern and central regions of South Africa. Monoxenous and heteroxenous helminths each spend part of their life cycle in the external environment and are therefore affected by microclimatic conditions (Mas-Coma et al. 2008; Nielson et al. 2007). Although the survival and distribution of free-living stages of both monoxenous and heteroxenous taxa will benefit from more favourable climatic conditions (precipitation and temperature) it does seem that the pattern is more complex for heteroxenous taxa. The eastern and central regions of South Africa are characterised by higher mean annual precipitation compared to the more xeric western regions (Cramer and Hoffman 2015). Precipitation facilitates soil moisture, plant growth and seed production (Cramer and Hoffman 2015). In South Africa, in particular, these conditions have been found to be important for insect abundance and diversity (Procheş and Cowling 2007; Botha et al. 2016). Precipitation is also an important driver for arthropod taxa that can act as intermediate hosts for heteroxenous

helminths (Anderson 2000; Georgiev et al. 2006; Dybing et al. 2013). For example, ambient temperature and precipitation have been shown to influence the diversity and abundance of mites (Krasnov et al. 2008). In addition, it was found that environmental conditions can potentially influence the infection of intermediate hosts by heteroxenous taxa (Shostak 2014). It is therefore not surprising that all of the heteroxenous nematodes and some of the cestodes were prevalent and abundant in areas where climate and vegetation are conducive to sustain large and diverse arthropod populations. Further, our current knowledge concerning the intermediate host spectrum of both nematode and cestode species in small mammals is too scant to judge if differences in the use of arthropod taxa might account for observed differences in geographic distribution.

Variation in climatic conditions can also explain the difference in the number of helminth species recorded in *Rhabdomys* spp. in the three seasonal localities. Springbok (GPR) is situated in the Succulent Karoo biome, which is characterised by predominantly shrub-land dominated by leaf-succulents or deciduous-leafed woody perennial dwarfs (Milton et al. 1997; Mucina and Rutherford 2006) and a low annual rainfall of 106 mm (Cowling et al. 2004a). In contrast, Stellenbosch (MBR) is situated in the Fynbos biome which is characterised by predominantly sclerophyllous and microphyllous shrubs and higher annual rainfall of 673 mm (Mucina and Rutherford 2006). At Springbok only two helminth species were recorded in *R. pumilio* and in low abundance, while six helminth species were recorded in *R. pumilio* at Stellenbosch. A similar species richness (n = 6) was recorded in *R. dilectus* at the Pretoria locality (KPR), which falls within the Savanna biome and receives a mean annual rainfall of 573 mm (Mucina and Rutherford 2006). Previously Froeschke et al. (2010) established that there is a significant positive association between nematode infection (burden and number of species) in *R. pumilio* and rainfall and humidity along a south-north climate gradient in South

Africa and Namibia. Our results, in addition to these findings, strongly suggest that climate, specifically rainfall combined with vegetation influences helminth burden and species richness.

Helminth infections varied seasonally at each of the three sampling localities. In general, both *R. pumilio* and *R. dilectus* demonstrated significantly higher helminth abundance and prevalence (as well as species richness for *R. pumilio* at MBR and *R. dilectus*) in the months following the wet season: summer in the case of *R. pumilio* (winter rainfall region) and winter for *R. dilectus* (summer rainfall region). The transition periods between wet and dry seasons (spring and autumn) are characterised by milder daytime temperatures (Musina and Rutherford 2006), which will further benefit free-living stages of helminth species and intermediate hosts. This suggestion is supported by a higher abundance and prevalence of the heteroxenous *M. weissi* during winter and spring in *E. myurus* trapped in the eastern summer rainfall region of South Africa (Lutermann et al. 2015). The latter authors deliberate the observed seasonal pattern as being generated by an accumulation of *M. weissi* with increased host age after the breeding season in summer, or as due to availability of infected intermediate hosts. In support of the present study, tick abundances (larvae and nymphs) were higher on *R. pumilio* during spring and summer compared to winter and autumn in the western winter rainfall region of South Africa (Matthee et al. 2007).

In conclusion, helminth species vary in host and geographic range and season. The study confirms the large diversity of helminth species associated with murid rodents and provides base-line data on possible host preferences. Because of the obvious patchiness of certain species, overall diversity may be underestimated if the sampling is not spatially extensive. This also means that local helminth faunas are expected to be rather different with respect to species composition. Spatial distribution maps identified taxon-specific variation in geographic extent

that is possibly driven by precipitation and vegetation cover. Our overall results also accentuate the scant published information currently available on the helminths associated with rodents, shrews and sengis in South Africa. Based on the novelty of the data it is essential that future research should concentrate on an increased and equal sampling effort to attain a more diverse range of host species.

Chapter 3

Helminth parasitism in two closely related South African rodents: abundance, prevalence, species richness and impinging factors

**Parasitology Research 116:1395–1409*

Introduction

All free-living animals are exploited by at least one parasite species and, in many cases, harbour an array of parasites. In particular, rodents are hosts for a large number of helminth taxa representing acanthocephalans, trematodes, cestodes and nematodes, with the latter two being the most common (Morand et al. 2006). These parasites have diverse life histories, modes of transmission and life cycles. Helminths have dual environments and are influenced by factors associated with both the host *per se* and the external environment. For example, the role of host-related factors is manifested in the similarity of helminth assemblages and infection levels among closely related host species within a geographic region (Poulin and Mouillot 2003, 2004; Mouillot et al. 2006). This is because a shared evolutionary history between closely related taxa generally results in more similar morphological (e.g. body size) physiological features) and ecological characteristics (e.g. diet) (de Graaff 1981; Skinner and Chimimba 2005). Host-associated ecological factors such as habitat use, nest type, population density and interaction with conspecifics, rather than the phylogenetic background, also influence the establishment and persistence of helminth assemblages (Lile 1998; Krasnov et al. 2008; Lutermann and Bennett 2012; Bordes et al. 2013; Moreno et al. 2013; Froeschke and Matthee 2014). On the other hand, helminth assemblages are affected by the physical environment (both biotic and abiotic) surrounding their hosts. For example, prevalence and abundance of strongyloid nematodes in the murid *Rhabdomys pumilio* (Sparrman) was positively associated with higher percentage vegetation cover (Froeschke and Matthee 2014). Similarly, it was found that habitat type (e.g., natural as opposed to transformed) and seasonal rainfall significantly affected the helminth abundance and species richness in rodents from Lao PDR (Pakdeenarong et al. 2014).

The rodent genus *Rhabdomys* Thomas is endemic to southern Africa. In South Africa, the genus is regionally wide-spread and locally abundant, occurring across the country (Skinner and Chimimba 2005). Skinner and Chimimba (2005) recognise two morphologically similar species, *R. pumilio* and *R. dilectus* (de Winton), with an estimated divergence time of ca. 3 Ma (Rambau et al. 2003). Despite close phylogenetic relatedness, the two species differ considerably in body size, sociality, habitat preference and geographic distribution. *Rhabdomys pumilio* is larger (males 214-216 mm and females 202-225 mm total length) than *R. dilectus* (males 170-200 mm and females 171-196 mm total length) (Skinner and Chimimba 2005). *Rhabdomys pumilio* forms social groups comprising 8-30 adult individuals (2-4 breeding females) with males and females having overlapping home ranges of similar size (Schradin and Pillay 2005a). This species occurs in the winter-rainfall western region of South Africa where the vegetation is mainly characterised by low-medium shrubs (Fig. 3.1). The region is characterised by a rainfall gradient with a less xeric southern part (250-600 mm per annum) and more xeric central and northern parts (< 250 mm p.a.) (Mucina and Rutherford 2006). In contrast, *R. dilectus* is solitary with adult males generally having larger home ranges compared to females (Dufour et al. 2015). This species prefers the mostly mesic eastern regions of South Africa where the main vegetation type is grass, though a combination of grass and shrub vegetation is found in the southern part of the distributional range (Mucina and Rutherford 2006) (Fig. 3.1). In this region, rainfall is predominantly summer to all-year (618-812 mm p.a.) (Joe Matsapola, personal communication, 2015). Both species are opportunistic omnivores. Major food items are herbage and seeds, and arthropods form part of the diet during most of the year (Perrin and Curtis 1980; Skinner and Chimimba 2005). As in many other murid rodents, coprophagy has been observed in *Rhabdomys* spp.; it is believed to help substitute mineral and protein levels and to assist in the population of the gastrointestinal flora of the young (Neville Pillay, personal communication, 2016). Thus, the two *Rhabdomys* species

present an excellent model to investigate the role of host ecology and behaviour as well as environmental conditions on helminth assemblages in closely related host species.

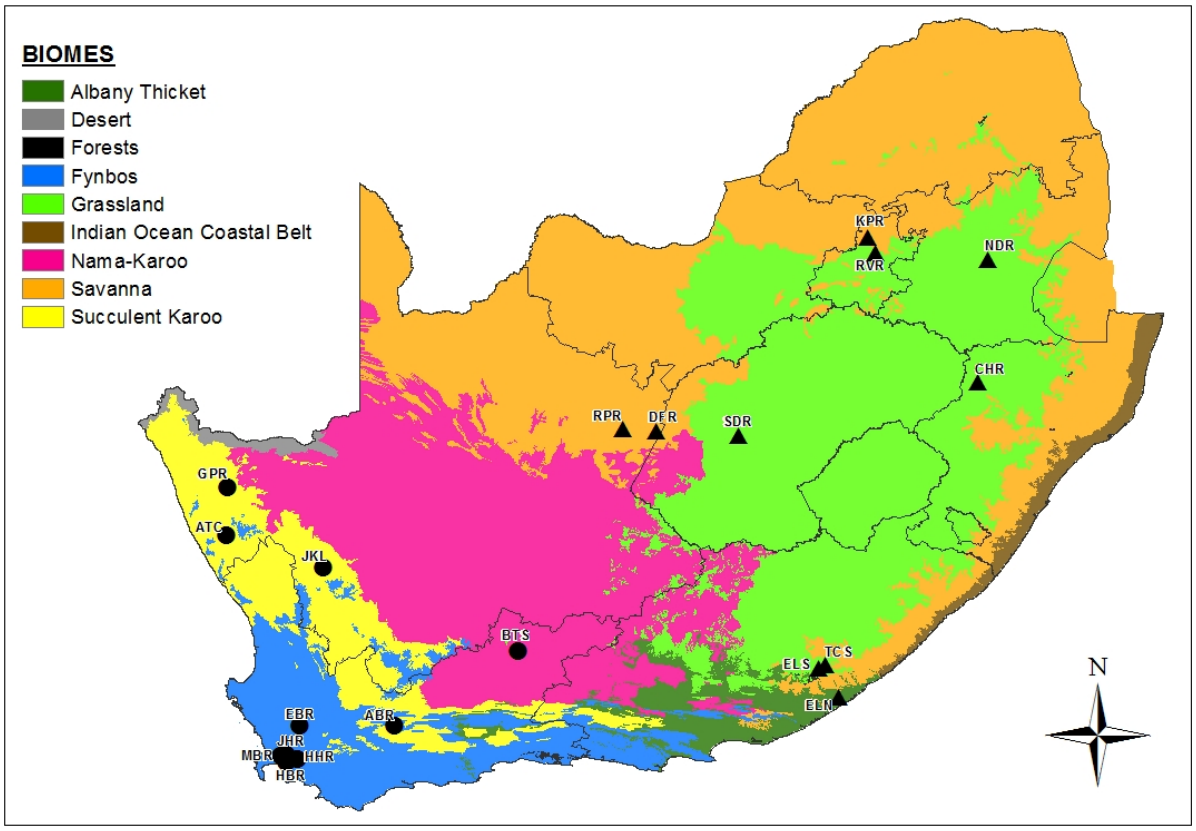


Fig. 3.1 Biome regions of South Africa, according to Mucina and Rutherford (2006) with study localities, Carolina (NDR); Pretoria site 1 (KPR); Pretoria site 2 (RVR); Springbok (GPR); Kimberley site 1 (DFR); Kimberley site 2 (RPR); Bloemfontein (SDR); Newcastle (CHR); Matjiesfontein (ABR); Stellenbosch site 3 (MBR); Stutterheim site 1 (ELS); Stutterheim site 2 (TCS); Beaufort West (BTS); Loeriesfontein (JKL); Garies (ATC); Somerset West (HBR); Stellenbosch site 2 (JHR); Stellenbosch site 1 (HHR); Wellington (EBR); East London (ELN). Triangles indicate *Rhabdomys dilectus* localities and dots *R. pumilio*.

Here, we studied the effects of host-related and environmental factors on helminth infection levels in the two species. Firstly, we asked whether patterns of helminth infection differ between *R. pumilio* and *R. dilectus*. Secondly, if yes, then whether the main reason for this difference was between-host difference in social structure or between-host difference in environmental conditions of the habitat that they occupy. On the one hand, among-individual interactions within groups of social species likely lead to higher transmission levels of those monoxenous helminths in which hosts are not merely infected by ingesting contaminated food,

but in which ingestion of eggs is also facilitated through coprophagy or grooming (Taffs 1976; Anderson 2000; Carlberg and Lang 2004). Furthermore, social animals also tend to have permanent nests and smaller home ranges compared to solitary species (Schradin 2006). The latter thus may be exposed to a higher density of helminths merely as a result of high probability of encounter. Consequently, we predicted that if the difference in social structure between the two species results in difference in pattern of helminth infection, then the effect of sociality would be manifested in the group-living *R. pumilio* exhibiting lower helminth species diversity but higher parasite burdens compared to the solitary *R. dilectus* (Arneberg 2002).

On the other hand, climate and vegetation may directly influence the free-living stages of parasitic helminths (Boomker et al. 2000; Turner and Getz 2010; Dybing et al. 2013). In South Africa, the mesic eastern region presents favourable conditions of precipitation and humidity for free-living stages of parasitic helminths, leading to higher survival and transmission rates, whereas xeric conditions would cause desiccation of these stages, resulting in lower parasite abundance. Consequently, if differences in environmental conditions determine differences in patterns of helminth infections, then we would expect to not only find lower helminth diversity but also lower helminth burdens in predominantly xeric *R. pumilio* than in predominantly mesic *R. dilectus*. Furthermore, environmental conditions can influence arthropod diversity and consequently the presence of heteroxenous helminths (e.g. cestodes and some nematodes) due to their dependence on arthropods as intermediate hosts for the completion of their life cycles. One would thus expect to find heteroxenous helminths to be more prevalent and abundant in areas where climate and vegetation are conducive to sustain large and diverse arthropod populations, such as the mesic regions inhabited by *R. dilectus*. In addition, the higher vagility seen in the solitary *R. dilectus* would likely lead to increased encounters with arthropods, when compared to *R. pumilio*. The effect of environmental conditions on helminths can further be

examined within *R. pumilio*. This rodent occurs in both the Fynbos and Succulent Karoo biomes within the western region (Skinner and Chimimba 2005). As mentioned above, lowland Fynbos is characterised by a higher average rainfall than the Succulent Karoo. In addition, the vegetation in the Fynbos is predominantly sclerophyllous and microphyllous shrubs, with grasses more commonly found in the eastern region of the biome (Cowling et al. 2004b). In contrast, the Succulent Karoo is predominantly shrub-land dominated by leaf-succulents or deciduous-leafed woody perennial dwarfs (Milton et al. 1997; Cowling et al. 2004b; Mucina and Rutherford 2006). Previously it was found that there is a significant positive association between nematode infection (burden and number of species) in *R. pumilio* and rainfall and humidity along a south-north climate gradient (Froeschke et al. 2010). We therefore analysed the difference in helminth burdens of *R. pumilio* between these two biomes and predicted higher helminth infection levels in *R. pumilio* living in the Fynbos compared to the Succulent Karoo.

Host-related factors such as gender and reproductive state likely play a role in the level of helminth infection in both *Rhabdomys* species. Reproductively active males are thought to be more prone to parasite infection due to larger body size, higher vagility and lower immunity due to high levels of testosterone when compared to reproductively active females and/or non-reproductive individuals (Folstad and Karter 1992; Arneberg 2002; Moore and Wilson 2002; Wirsing et al. 2007). Reproductively active females, and especially pregnant and lactating females, should be vulnerable to parasitism due to physiological constrains brought about by gestation, lactation and parental care when compared to non-reproductive females (Lo and Shaner 2015). In other words, we expected higher levels of helminth infection in (a) males than females and (b) reproductively active than non-reproductive individuals.

Research on the helminths associated with small mammals and specifically the ecological factors that drive helminth diversity and infection levels is sparse in southern Africa. While several comprehensive studies have been conducted in North and Western Africa [e.g., Egypt, Senegal and Tunisia (Behnke et al. 2000, 2004; Fichet-Calvet et al. 2003; Barnard et al. 2003; Brouat et al. 2007)], most research in southern Africa is restricted to taxonomic records in South Africa (Ortlepp 1939; Collins 1972). Studies on the environmental factors that shape helminth infections in small mammals in southern Africa have only recently been carried out (Froeschke et al. 2010; Froeschke and Matthee 2014). However, these studies focused mainly on nematodes in a single host species, *R. pumilio*, in the western biomes of South Africa and Namibia and are thus not necessarily representative of helminth infection affecting members of the genus *Rhabdomys* in South Africa as a whole.

Materials and methods

Sampling localities and study design

Rodents were trapped at 20 localities in several biomes of South Africa during 2004-2014 (Fig. 3.1). Mean annual rainfall varied among the biomes with 619.1 mm, 626.3 mm and 812.2 mm recorded for the Grassland, Savanna and Albany Thicket, respectively, while 224.2 mm, 267.8 mm and 505.5 mm was recorded for the Succulent Karoo, Nama Karoo and Fynbos, respectively. Rodents were mainly trapped during spring-summer (when they are the most active and abundant; Skinner and Chimimba 2005), with seasonal trapping conducted at 3 localities (Table 3.1).

Animals were captured using Sherman-type live traps, baited with a peanut butter - oats mixture. Traps were set out in 3-5 lines (50-100 m length) with 10 m distance between traps. Traps were checked twice daily and closed during the heat of the day (11:00-15:00) in summer

and the cold nights (17:00-08:00) in winter. Adult animals (*R. pumilio* or *R. dilectus*) were placed in plastic bags and euthanized by intraperitoneal injection with Sodium Pentobarbitone (200 mg/kg). All visibly juvenile *Rhabdomys* spp. and non-target species were identified (Apps 2000; Stuart and Stuart 2007; Cillié 2011) and released at the capture site. Each adult individual was sexed, weighed, measured (snout-to-vent and tail length) and its reproductive state was recorded. Reproductive state of females was assessed visually as either having a perforated vagina (reproductively active) or not and that of reproductive males as being visibly scrotal (reproductively active) or not.

Helminth recovery and identification

The gastrointestinal tract (GIT) from and including the oesophagus to the anus was removed, opened and stored in 70% ethanol. All helminths (total counts) were recovered from the stomach, small intestine and large intestine including the caecum and the number of nematodes and cestodes were logged (Haukisalmi and Henttonen 2001). Identification was based on relevant keys and descriptions of various authors. Voucher specimens of each species have been deposited in the National Collection of Animal Helminths at the ARC-Onderstepoort Veterinary Institute, South Africa (accession number of nematodes: S/2016/38) and the Finnish Museum of Natural History, Luomus, Finland (accession numbers of cestodes: KN 3646-KN 3668).

Data analysis

We considered patterns of helminth infection in the two host species on an individual as well as on a population level. On an individual level, we tested for the effect of host species (*R. dilectus* versus *R. pumilio*), sex and reproductive state on helminth incidence (see below), the number of helminth individuals and species in a host individual, applying (a) generalized linear

mixed-effects models (GLMM) with binomial error and logit-link function, using package "lme4" (Bates et al. 2015) for incidence, and (b) linear mixed-effects models (LME), using package "nlme" (Pinheiro et al. 2016) for the remaining dependent variables, implemented in R 3.3.0 statistical environment (R Core Team 2016). We included interactions between (a) host sex and reproductive state and (b) host species and sex in the models because (a) the effect of reproductive state on parasite abundance and/or species richness could be sex-dependent (Ferrari et al. 2004; Ribas and Casanova 2005; Wirsing et al. 2007; Krasnov et al. 2012) and (b) gender bias in parasite abundance and/or species richness could differ between solitary and social species (Bordes et al. 2007). The incidence variable was dichotomous and took a value of either 1 or 0 if an individual was either infected with at least one helminth or not infected at all, respectively. In the analyses of the number of helminth individuals and species harboured by a host individual, only individuals infected by at least one helminth individual were included. To test for the effect of biome [the Fynbos versus the Succulent Karoo (including the Nama Karoo)] on the number of nematode individuals and species (only a few cestode individuals were found in the Fynbos) harboured by an individual *R. pumilio*, we applied LME with sex and reproductive state of a host as well as interaction between these two factors as fixed effects.

To investigate patterns of helminth infection on a population level, we calculated prevalence of infection (separately for nematodes and cestodes) as the proportion of infected host individuals of each species, sex and reproductive state for sampling localities and periods in which at least three animals of a given cohort were captured. Then, we tested for the effect of species, sex and reproductive state on prevalence (separately for nematodes and cestodes) using LME and including interactions between explanatory variables as described above. In addition, we calculated prevalence for monoxenous nematodes that are transmitted via free-living stages

in the environment and those that can also be transmitted through coprophagy or grooming. We tested for differences in the prevalence of nematodes with the two transmission strategies between the two *Rhabdomys* species using LME, with host species and transmission strategy as well as interaction between them as explanatory variables. We also calculated prevalence of monoxenous and heteroxenous nematodes for *R. dilectus* (*R. pumilio* did not harbour heteroxenous nematodes) and tested whether these two helminth groups differed in prevalence using LME. Dependent variables were either log- (number of helminth individuals and species) or angular-transformed (prevalence) prior to analysis. Sampling locality was included as a random factor in each model because multiple individuals were captured in the same locality. Goodness-of-fit of each model was tested against a respective intercept-only model using ANOVA. We used Tukey's HSD test for multiple comparisons implemented in package "lsmeans" for R (Lenth 2016). We also calculated the degree of aggregation of nematodes and cestodes (separately) as *k* parameter of negative binomial distribution (see details in Taylor et al. 1979) using the moment estimate of Elliott (1977), corrected for sample size as $k = (M^2 - V/n)/(V - M)$, where M is mean parasite abundance, V is variance of parasite abundance and n is host sample size. We calculated the degree of aggregation only for sampling sites where at least five rodents were captured of which at least one was infected with either nematodes or cestodes. To test whether the degree of parasite aggregation among individual hosts differs between the two host species, we used Mann-Whitney U test.

Results

A total of 778 individual rodents were captured (486 *R. pumilio* and 292 *R. dilectus*) (Table 3.1). The small mammal species richness differed between sites, however *Rhabdomys* spp. were the most abundant rodent species in most localities.

Between-host effects

A total of 11 nematode and 5 cestode species were found in *R. pumilio* and 19 nematode and 7 cestode species were found in *R. dilectus* (Table 3.2). The two rodent species shared 8 helminth species (5 nematodes and 3 cestodes) (Table 3.2). The most abundant and prevalent species harboured by *R. pumilio* was *Heligmonina spira* (285.41 ± 28.49 and 46.3%, respectively), whereas *Neoheligmenella* sp. was the most abundant (24.55 ± 4.29) and *N. capensis* was the most prevalent (36.99%) species in *R. dilectus*. Mean abundance of cestode species was relatively low in both hosts and their prevalence was below 10%.

Table 3.1 Localities and biomes sampled and number of individuals collected for each of two *Rhabdomys* spp. (*R. dilectus* and *R. pumilio*) in South Africa, including relative *Rhabdomys* spp. density and total number of small mammal species trapped at the locality in parenthesis.

Rodent species and locality name	Code	GPS Co-ordinates South	GPS Co-ordinates East	Biome	Sample size	Relative *Rhabdomys* spp. density (number small mammal species)
R. dilectus						
Carolina	NDR	26.000	30.080	Gl	5	0.45 (5)
Pretoria site 1	KPR	25.635	28.167	Sv	97*	0.23 (7)
Pretoria site 2	RVR	25.897	28.294	Gl	24	0.96 (4)
Bloemfontein	SDR	28.823	26.089	Gl	19	1.90 (3)
Newcastle	CHR	27.964	29.922	Gl	23	2.08 (5)
Stutterheim site 1	ELS	32.490	27.489	Gl	30	4.00 (2)
Stutterheim site 2	TCS	32.547	27.368	Gl	25	3.09 (2)
Kimberley site 1	DFR	28.742	24.772	Sv	32	3.62 (1)
Kimberley site 2	RPR	28.716	24.230	Sv	15	1.70 (5)
East London	ELN	33.005	27.703	At	22	2.10 (1)
Total					**292**	
R. pumilio						
Springbok	GPR	29.660	17.893	Sk	120*	0.79 (1)
Loeriesfontein	JKL	30.950	19.433	Sk	31	3.62 (2)
Garies	ATC	30.423	17.884	Sk	27	0.49 (1)
Beaufort West	BTS	32.271	22.565	Nk	15	1.54 (2)
Matjiesfontein	ABR	33.467	20.584	Fb	27	1.83 (6)
Stellenbosch site 1	HHR	34.002	19.022	Fb	42	5.72 (1)
Stellenbosch site 2	JHR	33.931	18.854	Fb	40	2.02 (1)
Stellenbosch site 3	MBR	33.949	18.766	Fb	118*	1.59 (4)
Somerset West	HBR	34.055	18.844	Fb	34	3.57 (1)
Wellington	EBR	33.480	19.065	Fb	32	0.60 (1)
Total					**486**	

*Localities where animals were trapped on four occasions in a year.
Gl = Grassland, Sv = Savanna, At = Albany Thicket, Sk = Succulent Karoo, Nk = Nama / Succulent Karoo, Fb = Fynbos.

Table 3.2 Species, transmission strategy, prevalence (%) and mean abundance (± SE) (MA) of nematodes and cestodes infecting *Rhabdomys dilectus* and *R. pumilio.*

Higher taxon and species	Life cycle	*R. dilectus*		*R. pumilio*	
Nematodes		Prevalence (%)	MA (± SE)	Prevalence (%)	MA (± SE)
Paralibyostrongylus sp.	Direct	6.85	0.39 ± 0.13	0	0
Paralibyostrongylus sp. 1	Direct	0	0	6.38	0.26 ± 0.07
Trichostrongylus probolurus (Railiet, 1896)	Direct	0	0	9.47	2.59 ± 0.92
Trichostrongylus sp.	Direct	10.27	4.56 ± 1.29	0.21	0.02 ± 0.02
Nematodirus sp.	Direct	0	0	0.82	0.11 ± 0.09
Heligmonina boomkeri Durette-Desset and Diginani, 2005	Direct	8.56	0.33 ± 0.09	0	0
Heligmonina spira (Ortlepp, 1939)	Direct	18.84	19.40 ± 4.15	46.30	285.41 ± 28.49
Heligmonina sp.	Direct	0.34	0.003 ± 0.003	0	0
Heligmonina sp. 3	Direct	0	0	0.62	0.05 ± 0.05
Neoheligmonella capensis (Ortlepp, 1939)	Direct	36.99	8.62 ± 1.71	27.98	5.45 ± 0.64
Neoheligmonella sp.	Direct	26.37	24.55 ± 4.29	0	0
Neoheligmonella sp. 1	Direct	0	0	5.56	0.46 ± 0.15
Syphacia obvelata (Rudolphi, 1802)	Direct	0.34	0.003 ± 0.003	0	0
Syphacia sp.	Direct	29.79	10.15 ± 2.15	21.40	6.36 ± 1.62
Aspiculuris shikoloueta Inglis, Harris and Lewis, 1990	Direct	0	0	5.97	1.27 ± 0.38
Trichuris muris (Schrank, 1788)	Direct	1.03	0.01 ± 0.01	0.41	0.004 ± 0.003
Trichuris sp.	Direct	0.34	0.003 ± 0.003	0	0
Abbreviata sp.	Indirect	0.68	0.05 ± 0.05	0	0
Protospirura muricola Gedoelst, 1916	Indirect	2.74	0.17 ± 0.08	0	0
Protospirura numidica Seurat, 1914	Indirect	3.08	0.44 ± 0.35	0	0
Protospirura sp.	Indirect	0.68	0.01 ± 0.01	0	0
Ascarops strongylina (Rudolphi, 1819)	Indirect	0.34	0.02 ± 0.02	0	0
Ascarops sp.	Indirect	1.03	0.12 ± 0.11	0	0
Streptopharagus lerouxi Quentin, 1965	Indirect	0.34	0.003 ± 0.003	0	0
Mastophorus muris (Gmelin, 1790)	Indirect	0.68	0.08 ± 0.07	0	0

Cestodes					
Inermicapsifer sp.	Indirect	5.82	0.14 ± 0.04	0	0
Meggittina baeri Lynsdale, 1953	Indirect	5.14	0.64 ± 0.23	4.53	0.35 ± 0.12
Skrjabinotaenia sp.	Indirect	0	0	1.03	0.03 ± 0.01
Skrjabinotaenia sp. 4	Indirect	1.37	0.04 ± 0.02	0.82	0.05 ± 0.03
Skrjabinotaenia sp. 6	Indirect	0	0	1.44	0.02 ± 0.01
Raillietina trapezoides (Janicki, 1904)	Indirect	5.82	0.65 ± 0.22	7.61	1.65 ± 0.37
Rodentolepis cf. *fraterna* (Stiles, 1906)	Indirect	1.37	0.02 ± 0.01	0	0
Rodentolepis sp.	Indirect	4.79	0.21 ± 0.08	0	0
Rodentolepis microstoma (Dujardin, 1945)	Indirect	1.71	0.11 ± 0.07	0	0

Incidence of nematode infection was significantly higher in *R. dilectus* than in *R. pumilio* as well as in reproductively active than in non-reproductive individuals, although it did not differ between males and females (Table 3.3). The number of nematode individuals harboured by an individual rodent was also similar in males and females and was significantly higher in reproductively active animals, but did not differ between *R. pumilio* and *R. dilectus* (Table 3.3; Fig. 3.2). In addition, the interaction between host sex and reproductive state appeared significant (Table 3.3). This significance was manifested in that the difference between reproductive and non-reproductive rodents in the number of nematodes they harbour was more pronounced in males than in females (Tukey's HSD tests, t-ratio = 6.82 versus 3.01, respectively). Nematode species richness in an individual host was affected by host reproductive state only, with reproductive animals harbouring richer nematode assemblages (Table 3.3, Fig. 3.2). Cestode incidence was significantly higher in reproductively active than in non-reproductive rodents and did not differ between either host species or host gender, whereas cestode abundance and species richness were not affected by any host-related factor or their interactions (Table 3.3). Models of the effects of host-related variables on nematode incidence, abundance and species richness but not prevalence fitted data significantly better than the intercept-only models, whereas this was not the case for models involving cestodes (Table 3.4).

Table 3.3 Summary of (a) generalized linear mixed-effects models (GLMM) with binomial error for incidence (NI for nematodes and CI for cestodes) and (b) linear mixed-effects models (LME) for abundance and species richness (NAB and NSR, respectively, for nematodes and CAB and CSR, respectively, for cestodes) of helminths in rodent hosts as affected by host species identity (HS; *Rhabdomys pumilio* versus *R. dilectus*), host sex (Sex; female versus male) and host reproductive state (HRS; reproductively active versus non-reproductive). Reference levels for independent variables were *R. dilectus* for HS, female for Sex and non-reproductive for HRS. *z*-values are presented for GLMM and *t*-values are presented for LME.

Dependent variable	Explanatory variable	Coefficient estimate ± SE	*z/t*	*p*
NI	HS	-3.29 ± 1.18	-2.79	0.004
	Sex	0.003 ± 0.51	0.006	0.99
	HRS	0.76 ± 0.36	2.12	0.03
	Sex x HRS	-0.56 ± 0.47	-1.18	0.23
	Sex x HS	0.81 ± 0.49	1.66	0.10
NAB	HS	0.21 ± 0.29	0.71	0.48
	Sex	-0.21 ± 0.14	-1.53	0.12
	HRS	0.31 ± 0.10	3.01	0.002
	Sex x HRS	0.33 ± 0.14	2.44	0.01
	Sex x HS	0.09 ± 0.12	0.78	0.43
NSR	HS	-0.04 ± 0.05	-0.84	0.41
	Sex	-0.01 ± 0.02	-0.32	0.75
	HRS	0.04 ± 0.02	2.33	0.02
	Sex x HRS	0.02 ± 0.02	1.01	0.31
	Sex x HS	0.01 ± 0.02	0.71	0.47
CI	HS	-0.70 ± 2.36	-0.30	0.77
	Sex	0.45 ± 1.07	0.42	0.67
	HRS	1.44 ± 0.62	2.32	0.02
	Sex x HRS	-1.14 ± 1.06	-1.07	0.28
	Sex x HS	0.13 ± 0.61	0.22	0.82
CAB	HS	0.28 ± 0.21	1.32	0.22
	Sex	0.11 ± 0.29	-0.02	0.98
	HRS	0.02 ± 0.17	0.36	0.71
	Sex x HRS	0.01 ± 0.29	-1.29	0.20
	Sex x HS	-0.20 ± 0.16	-0.25	0.80
CSR	HS	-0.02 ± 0.03	-0.59	0.56
	Sex	-0.01 ± 0.06	-0.03	0.97
	HRS	-0.03 ± 0.04	-0.76	0.44
	Sex x HRS	0.04 ± 0.06	0.59	0.56
	Sex x HS	-0.02 ± 0.03	-0.57	0.56

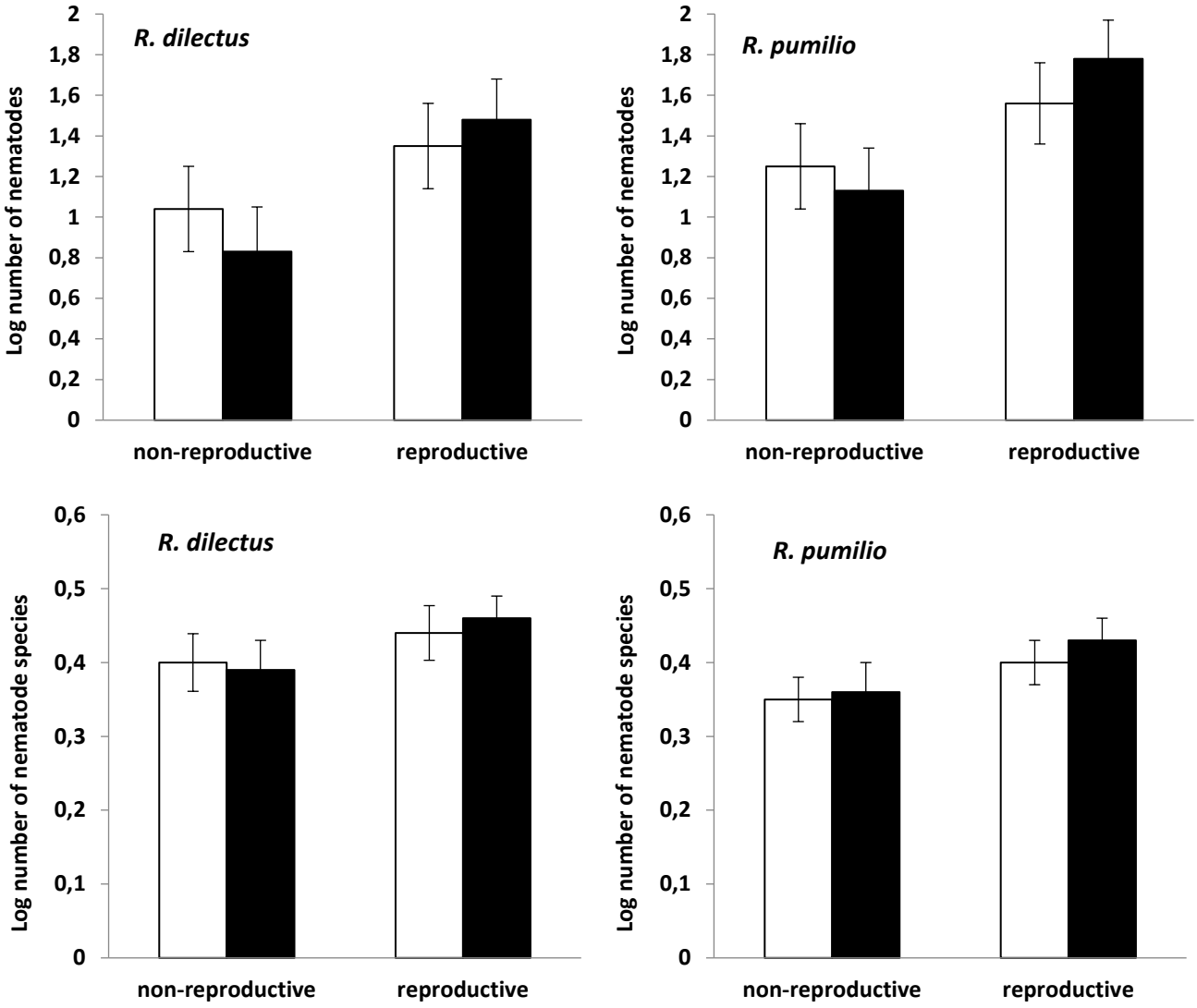


Fig. 3.2 Number (least-squares means of log-transformed values ± SE) of nematode individuals (upper panel) and species (lower panel) harboured by non-reproductive and reproductively active male (black) and female (white) *Rhabdomys dilectus* and *R. pumilio*.

Table 3.4 Comparisons of (a) generalized linear mixed-effects models (GLMM) with binomial error of prevalence and (b) linear mixed-effects models (LME) for abundance and species richness of helminths in *Rhabdomys pumilio* and *R. dilectus* as affected by host species identity, sex and host reproductive state with the respective intercept-only models. See Table 3.3 for abbreviations of the dependent variables of the models. AIC – Akaike Information Criterion (AICm – AIC of the model, AIC_0 – AIC of the intercept-only model), LL – log-Likelihood (LLm – LL of the model, LL_0 – LL of the intercept-only model), LR – log-Likelihood ratio. χ^2-values are presented for GLMM and LR values are presented for LME.

Dependent variable	AICm	AIC_0	LLm	LL_0	χ^2/LR	*p*
NI	652.4	656.9	-319.18	-326.46	14.56	0.01
NAB	1043.4	1089.7	-513.70	-541.88	56.35	< 0.001
NSR	-659.5	-644.2	337.86	325.08	25.56	< 0.001
CI	339.1	336.7	-162.54	-166.37	7.66	0.17
CAB	135.2	128.1	-59.58	-61.07	2.98	0.70
CSR	-208.4	-215.1	112.21	110.53	3.34	0.64

Prevalence of both nematodes and cestodes did not depend on either host species, sex or reproductive state or their interactions (Table 3.5). Moreover, models of the effect of host species identity, sex and reproductive state on helminth prevalence did not provide better fit to data than the intercept-only models (log-likelihood ratio = 6.74 for nematodes and log-likelihood ratio = 7.06 for cestodes; $p > 0.13$ for both).

Rhabdomys pumilio only harboured monoxenous nematodes. In contrast, *R. dilectus* harboured both monoxenous and heteroxenous nematodes, and the prevalence of the former was higher (1.09 ± 0.11) than that of the latter (0.45 ± 0.13) (LME, reference level for explanatory variable was heteroxenous life cycle, coefficient ± S.E. = 0.64 ± 0.17, $p = 0.004$; ANOVA, F = 14.28, $p = 0.04$). Looking at monoxenous nematodes, they were equally prevalent in both host species. Furthermore, nematodes that are transmitted through ingestion of free-living stages in the environment while foraging were significantly more prevalent than nematodes that can also be transmitted through coprophagy and/or grooming in both host species (Table 3.6; Fig. 3.3).

Table 3.5 Summary of linear mixed-effects models (LME) for prevalence of helminths (NP for nematodes and CP for cestodes) in *Rhabdomys pumilio* and *R. dilectus* as affected by host species identity, host sex and host reproductive state. See Table 3.3 for abbreviations of the dependent variables of the models and reference levels for independent variables.

Dependent variable	Explanatory variable	Coefficient estimate ± SE	*z/t*	*p*
NP	HS	-0.25 ± 0.22	-1.13	0.27
	Sex	0.07 ± 0.14	0.54	0.59
	HRS	0.11 ± 0.08	1.32	0.19
	Sex x HRS	-0.14 ± 0.12	-1.17	0.24
	Sex x HS	0.14 ± 0.12	1.22	0.22
CP	HS	-0.02 ± 0.03	-0.59	0.56
	Sex	-0.01 ± 0.06	-0.03	0.97
	HRS	-0.03 ± 0.04	-0.76	0.44
	Sex x HRS	0.04 ± 0.06	0.59	0.56
	Sex x HS	-0.02 ± 0.03	-0.57	0.56

Table 3.6 Summary of linear mixed-effects models for prevalence of monoxenous nematodes as affected by their different transmission strategies (TS, see text for explanations) and host species (HS). Reference levels for independent variables were (a) nematodes not transmitted via coprophagy and grooming for TS and (b*) Rhabdomys dilectus* for HS.

Dependent variable	Coefficient estimate ± SE	*t*	*p*
TS	-0.29 ± 0.09	-3.33	< 0.01
HS	0.05 ± 0.12	0.37	0.71
TS x HS	-0.14 ± 0.12	-1.10	0.28

The degree of nematode aggregation among host individuals did not differ significantly between *R. pumilio* and *R. dilectus* (Mann-Whitney U = 41.0, Z = 1.35, $p = 0.17$). The same was true for cestodes (Mann-Whitney U = 4.0, Z = 1.34, $p = 0.18$).

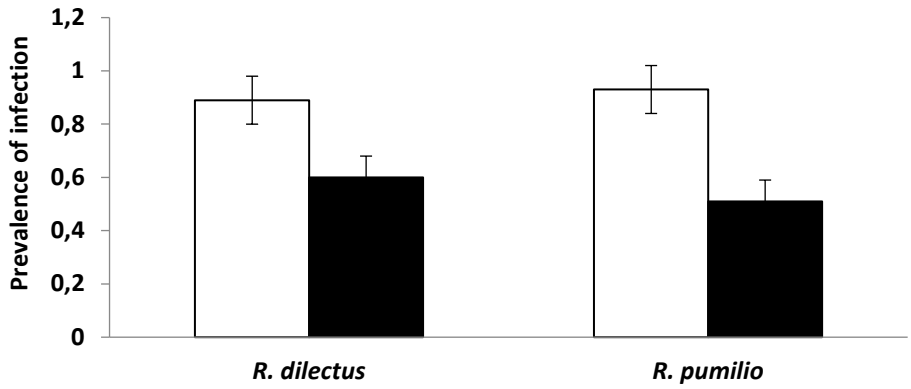


Fig. 3.3 Prevalence (least-squares means of angular-transformed values ± SE) of monoxenous nematodes transmitted through free-living stages in the environment (white) and those that can also be transmitted through coprophagy and grooming (black) in *Rhabdomys dilectus* and *R. pumilio.*

Between-biome within-R. pumilio effects

In *R. pumilio*, the number of nematode individuals and species in an individual host differed significantly between biomes, being higher in the Fynbos than in the Karoo region (Succulent and Nama Karoo biomes combined) (mean abundance was 498.42 ± 44.67 and 90.5 ± 1.45, respectively), and was affected by rodent reproductive state (higher in reproductively active individuals) (Table 3.7, Fig. 3.4). In addition, interaction between host sex and reproductive state was significant in the model of nematode numbers (Table 3.7). Significance of interaction was reflected by a more pronounced difference between reproductive and non-reproductive males than females (Tukey's HSD tests, t-ratio = 5.97 versus 2.41, respectively). Both models fitted data significantly better than intercept-only models (log-likelihood ratio = 55.82 for number of nematode individuals and log-likelihood ratio for number of nematode species = 37.10, $p < 0.001$ for both).

Table 3.7 Summary of linear mixed-effects models for the number of nematode individuals and species (NAB and NSR respectively) in individual *Rhabdomys pumilio* as affected by biome (B; Fynbos versus Succulent/Nama Karoo), host sex (Sex; female versus male) and host reproductive state (HRS; reproductively active versus non-reproductive). Reference levels for independent variables were Fynbos for B, female for Sex and non-reproductive for HRS.

Dependent variable	Explanatory variable	Coefficient estimate ± SE	*t*	*p*
NAB	B	-1.25 ± 0.19	-6.50	< 0.0001
	Sex	-0.19 ± 0.17	-1.31	0.26
	HRS	0.37 ± 0.15	2.41	0.01
	Sex x HRS	0.42 ± 0.20	2.10	0.03
NSR	B	-0.18 ± 0.03	-6.64	0.0002
	Sex	0.01 ± 0.03	0.29	0.76
	HRS	0.06 ± 0.02	2.31	**0.02**
	Sex x HRS	0.02 ± 0.03	0.58	0.56

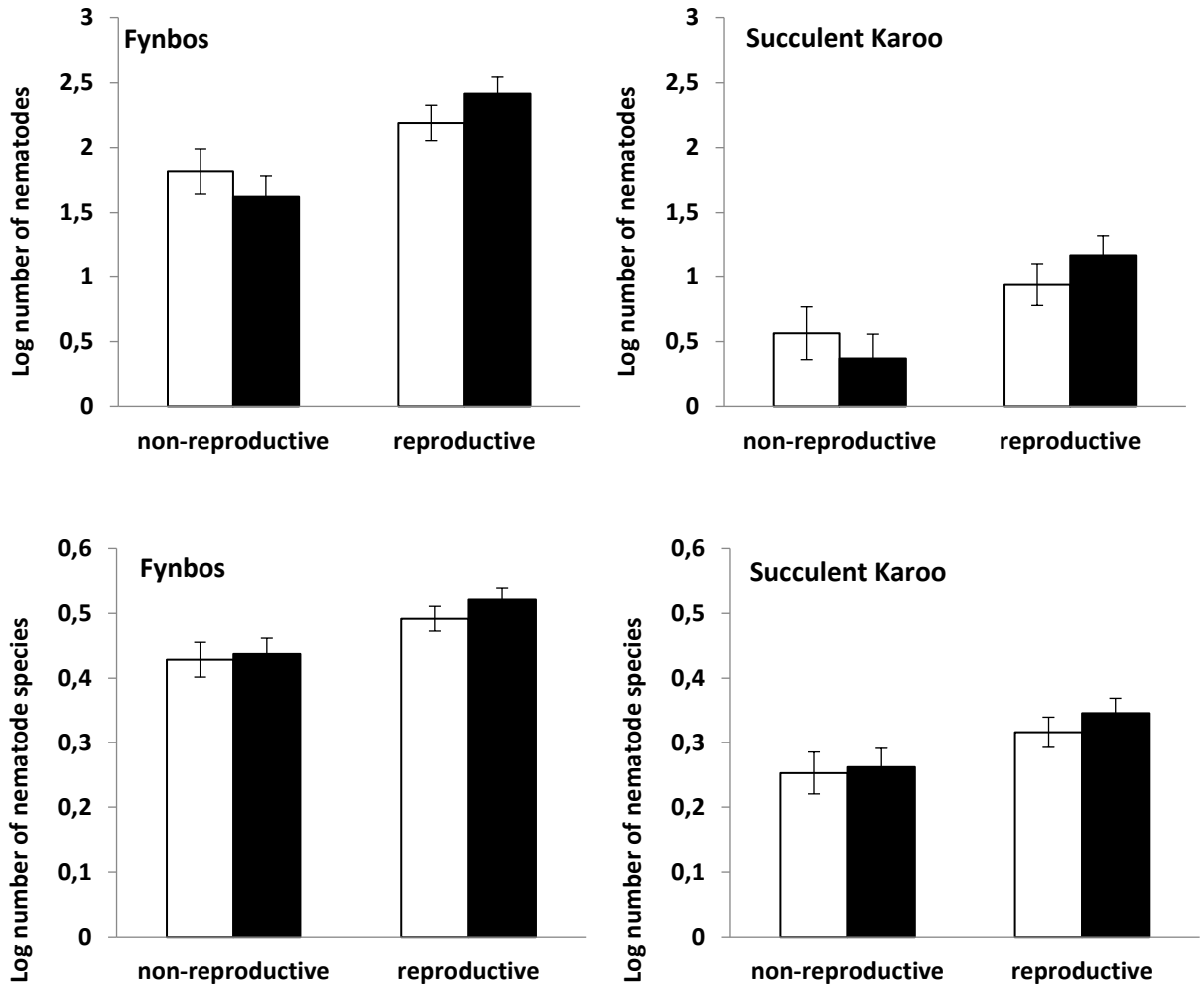


Fig. 3.4 Number (least-squares means of log-transformed values ± SE) of nematode individuals (upper panel) and species (lower panel) harboured by non-reproductive and reproductively active male (black) and female (white) *Rhabdomys pumilio* in Fynbos and Succulent Karoo.

Prevalence of cestodes differed within *R. pumilio* but distinctly between biomes, being very low at 2.39% in the Fynbos (only two species were recorded), and moderate at 26.94% in the larger Karoo region, where a total of four cestode species were recorded.

Discussion

In general, our results did not conform to any of our scenarios that would allow to distinguish between the effects of host sociality and environmental factors on the number of individuals and species of helminths harboured by spatially separate but closely related rodent species. However, differences in the incidence of nematode infection between *R. pumilio* and *R. dilectus* as well as differences in the number of nematode individuals and species between *R. pumilio* from the Fynbos and conspecifics from the Succulent/Nama Karoo suggested that the effect of environmental conditions on helminth infection is likely more important than the effect of sociality.

Five nematode species attained more than 10% prevalence in *R. dilectus*, whereas in the case of *R. pumilio* only three species belonged in this category. In addition, total nematode species richness was higher in *R. dilectus* than in *R. pumilio* (73% of all species recorded and 44%, respectively). Moreover, comparisons of nematode species that occurred in both rodent hosts indicated that the numbers and prevalence of several abundant nematode species were higher in *R. dilectus* than in *R. pumilio* (Table 3.2). However, and despite these differences, there were no significant differences in the number of nematode individuals and species harboured by individual hosts of either species. Nevertheless, nematode incidence did differ significantly between the two host species which supports our prediction that the probability of being infected differs between the *Rhabdomys* species, and was higher in the mesic habitat-dwelling *R. dilectus* compared to the predominantly xeric habitat-dwelling *R. pumilio*. This indicates that when a rodent individual is already infected, the number of helminths it harbours does not depend on its identity. Furthermore, the number of nematode species was higher in *R. pumilio* in Fynbos (10) compared to *R. pumilio* in the Karoo (3) and several of the most abundant nematode species in the Fynbos were more

abundant and prevalent (e.g. *Heligmonina* sp.: 473.40 ± 43.95, 76.79% and *Syphacia* sp.: 9.91 ± 2.64, 31.06%) compared to the most abundant species in the Karoo (*Aspiculuris shikoloueta*: 2.68 ± 0.86, 12.95% and *Syphacia* sp.: 0.96 ± 0.47, 7.77%). This finding provides stronger support for our prediction that the environmental conditions associated with the more mesic regions may be more favourable for the survival of free-living nematode stages. This also supports the results of an earlier study carried out along a climate gradient in South Africa and Namibia in which nematode species richness and abundance in *R. pumilio* was found to be positively associated with mean annual precipitation (Froeschke et al. 2010). A similar pattern was found for other host species in other regions. For example, a study on helminth prevalence in red foxes [*Vulpes vulpes* (Linnaeus)] in Western Australia reported a positive association between relative humidity and the longevity of infective free-living helminth stages (Dybing et al. 2013). In the present study, biomes located in the eastern part of South Africa generally receive higher mean annual rainfall compared to western biomes. Climatic factors such as mean annual precipitation and temperature significantly affects vegetation cover and plant growth forms with the result that the normalised difference vegetation index (which measures chlorophyll in vegetative cover) is higher in eastern biomes compared to western biomes (Cramer and Hoffman 2015). Vegetation height and cover decreases air and soil temperatures, probably due to the interception or reflection of solar radiation (Song et al. 2013). Lower vegetation cover and lower mean annual rainfall will result in higher soil temperatures and increased desiccation of free-living nematode stages, which explains why the nematode infection was significantly lower in *R. pumilio* in the Karoo compared to the Fynbos. Obviously, biome-related climatic conditions and vegetation (structure and plant growth forms) also affect arthropod communities (Procheş and Cowling 2007; Botha et al. 2016). Heteroxenous cestodes and nematodes, in turn, are dependent on a variety of arthropods as their primary

intermediate hosts (Morgan and Hawkins 1951; Yamaguti 1961; Ubelaker 1970; Kinsella 1991). Consequently, the effect of environmental conditions on helminth assemblages might be both direct (i.e., affecting free-living stages) and indirect (i.e., affecting intermediate hosts). Indeed, in the present study, *R. dilectus* harboured both monoxenous and heteroxenous nematodes, as well as cestodes. *Rhabdomys pumilio*, on the other hand, did not harbour any heteroxenous nematodes, but was nevertheless host to three genera of cestodes. One of the reasons behind these differences might be found in the suitability of various arthropods as intermediate hosts for different groups of helminths, and in turn, the suitability of a given habitat for certain arthropod intermediate hosts.

In this context, it is noteworthy that *Inermicapsifer* sp. (Anoplocephalidae s. l.) was present in *R. dilectus* but absent in *R. pumulio*. Oribatid mites are intermediate hosts of numerous anoplocephalid cestodes (Denegri 1993; Schuster et al. 2000; Georgiev et al. 2006). The abundance of these mites has been shown to be positively correlated with soil moisture content and organic matter (Mitchell 1978; Kim and Jung 2008), and Sanada and Aoki (1999) report diurnal migrations of infected mites from the soil to the top of grass leaves. Based on these characteristics, one might speculate that cestodes using oribatid mites as intermediate hosts find better conditions and likely attain a higher abundance and transmission rates in mesic eastern "grassy" habitats than in xeric western shrub habitats. Similarly, *Rodentolepis* spp. were present in *R. dilectus*, but not in *R. pumilio*. Hymenolepidids are known to use a wide range of arthropod hosts, such as e.g. coleopterans, dermapterans, orthopterans, siphonapterans and diplopods (see Georgiev et al. 2006). Interestingly, many of these also serve as intermediate hosts for the heteroxenous nematodes that were found in *R. dilectus* (see Anderson 2000). Hence the presence

of these helminth taxa in *R. dilectus* might be linked to the suitability of its more mesic environment to the survival and proliferation of certain groups of arthropods.

Generally speaking, however, our knowledge on the natural intermediate host spectrum of a given helminth species is scant and this is particularly true for catenotaeniids which comprised the majority of cestode species present in *R. pumilio*. To date, the life cycle of a single species, *Catenotaenia pusilla* (Goeze, 1782), within this family has been elucidated, reporting a tyroglyphid mite as intermediate host (see Georgiev et al. 2006), but nothing is known about catenotaeniids in Africa. The davaineid *Raillietina trapezoides* seems well adjusted to hosts in arid environments (Wertheim et al. 1986; Fichet-Calvet et al. 2003; Faleh et al. 2012) and was the most prevalent and abundant cestode in *R. pumilio* in the Karoo and the second most prevalent cestode in this host in the Fynbos. Ants that thrive in arid habitats and are intermediate hosts for some davaineids, amongst others *Railletina* spp. (O'Callaghan et al. 2003), might contribute to this phenomenon. Despite its obviously successful adaptation to dry habitats, *R. trapezoides* was, however, also recovered from *R. dilectus* in the mesic eastern regions of South Africa. Given the many shortcomings in our understanding of the life histories of cestodes parasitising rodents, it is difficult to explain why both prevalence as well as abundance of cestodes was distinctly higher in *R. pumilio* in the Karoo when compared to the Fynbos. Fires, such as those that form part of the specialised ecology of Fynbos vegetation, have been reported to decrease the abundance of soil microarthropods, due to destruction of vegetation and leaf litter and changes in soil pH and structure (Heyward and Tissot 1936; Kim and Jung 2008). They might similarly impact on a wider range of possible arthropod intermediate hosts. Torre et al. (2013) recorded a temporary absence of heteroxenous helminths in *Apodemus sylvaticus* (Linnaeus) following fires in Mediterranean

forests in Spain, and state that fires may have a long-lasting impact on invertebrate intermediate hosts. Clearly, any explanation as to the intriguing pattern found in the distribution of cestodes between the Fynbos and Karoo biome is at this stage rather speculative and warrants further investigation.

Although it does seem that environmental conditions provide stronger support for the patterns that were observed we cannot refute the role of host sociality and spatial behaviour in variation in helminth infections. Solitary species are often more vagile when compared to group-living species and as such host vagility could be an additional factor that can contribute to a higher contact rate with free-living nematode life stages and arthropods (Bordes et al. 2009). The solitary *R. dilectus* have significantly larger home ranges compared to social *R. pumilio* (Schradin and Pillay 2005a) and therefore have a greater chance of encountering more diverse vegetation types, with associated unique microclimatic conditions and diverse small mammal species. Another factor that can contribute to an increase in contact rate is the fact that *R. dilectus* seems to have a longer breeding season (seven months) compared to *R. pumilio* (three months) (Schradin 2005; Schradin and Pillay 2005a). In fact, our results strongly support the above scenarios in that *R. dilectus* harboured more nematode species than *R. pumilio*.

The role of spatial behaviour in facilitating helminth infections is commonly observed between reproductive and non-reproductive individuals (Folstad and Karter 1992; Moore and Wilson 2002; Waterman et al. 2013; Vandegrift and Hudson 2009), as well as in infection differences between male and female hosts (although it is not a universal rule; see Kiffner et al. 2013). Indeed, we found that the number of nematode individuals and species as well as probability of cestode

infection was significantly higher in reproductively active compared to non-reproductive rodents. This pattern was even more distinct for the number of nematodes harboured by reproductive versus non-reproductive males. An increase in home range size and dispersal from natal groups lead to higher vagility in males during the breeding season (Schradin and Pillay 2005a; Bordes et al. 2009). However, larger home ranges are not characteristic for males only as female *R. pumilio* have also been shown to increase their home range size with the on-set of the breeding season in the Succulent Karoo (Schradin and Pillay 2006). Despite rodents breeding in spring when abundance of high protein annual plants and insects forming the basis of their diet is high (Schradin 2005; Schradin and Pillay 2006), males and females nevertheless increase the volume and diversity of food to keep up with the energy demand (Perrin and Curtis 1980; Perrin et al. 2001). The resulting increase in exposure to infective stages may provide an additional explanation for the higher nematode infection in reproductively active individuals. Male rodents have the added costs of maintaining higher testosterone levels and display aggressive behaviour towards strangers encountered in their own home range during the breeding season (Schradin and Pillay 2004). This may further contribute to immune suppression, higher energy demands and increased food intake. A similar pattern was previously recorded by Moore and Wilson (2002) in a comparative mammal-host-parasite study where they showed that males suffered a sex-specific handicap linked with the production of immunodepressive androgens. In addition, Waterman et al. (2013) reported that male Richardson's ground squirrels [*Spermophilus richardsonii* (Sabine)] display high aggression during the mating season, suggesting a trade off investment in immunity for higher investment in reproduction, with a subsequent higher nematode burden.

Although the two *Rhabdomys* species differ in level of sociality and thus natal group size and frequency of intra-group contact (Schradin and Pillay 2005a; Skinner and Chimimba 2005; Schradin 2006), it seems that group-living did not have a significant effect on the presence of monoxenous nematodes with different modes of transmission. Specifically, nematodes that can be transmitted through coprophagy and/or grooming and eggs in the environment were equally prevalent in the two host species. This pattern contradicts our expectations that the group-living *R. pumilio* would harbour higher numbers of these parasites due to close body contact in the nest and during allogrooming events. A possible explanation is that the largest component of transmission of eggs could be due to ingestion of eggs that contaminate the environment, which would severely dilute the impact of grooming on overall transmission rates. This said, and possibly emphasising the effect of environment on helminth assemblages versus sociality, an interesting pattern emerged with regard to *A. shikoloueta*. This parasite did not occur in *R. dilectus*, but was present in *R. pumilio*. In the latter, it had a low prevalence and mean abundance in the Fynbos (1.37% and 0.32 ± 0.27, respectively), but a high prevalence and mean abundance in the Karoo (12.95% and 2.68 ± 0.86, respectively). While no information is available for *A. shikoloueta* itself, Anderson (2000) lists its congener *A. tetraptera* as probably transmitted through ingestion of contaminated food and coprophagy. In rodents and other small mammals, coprophagy is thought to be a means of substituting nutrient poor diets, with the added benefit of water conservation (Jameson 1981; Cranford and Johnson 1983; Kenagy et al. 1999). Hence, one might speculate that coprophagy in hosts in the Karoo, as behavioural adaptation to the arid climate and associated shortage in high quality food items, favours transmission of certain parasites.

To conclude, our results suggest that environmental factors such as precipitation and vegetation cover contribute strongly to differences in nematode and cestode infection between and within *Rhabdomys* species.

Chapter 4

Community structure of helminth parasites in two closely related South African rodents differing in sociality and spatial behaviour

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Introduction

Extensive studies of community structure in various taxa have shown that biological communities are often organized by certain rules (Hanski 1982; Patterson and Atmar 1986; Fox and Brown 1993; Gotelli and McCabe 2002), although other communities seem to be random species assemblages (Strong et al. 1979; Gotelli et al. 1997; Gainsbury and Colli 2003; Sanders et al. 2007; Vieira et al. 2014). One of the major questions in community ecology has thus been to understand what factors determine the occurrence, pattern and degree of non-randomness in community assemblies. A variety of factors have been reported to influence community structure. Among them, factors associated both with environmental conditions and factors acting within the community itself can be distinguished. In other words, community structure arises as interplay between the biotic and abiotic properties of the habitat in which a community occurs and the intrinsic properties of species composing this community as well as their interactions. For example, strong impact of local conditions on assemblage structure has been shown for treehole macroinvertebrate communities (Paradise et al. 2008); island plant communities (Lu et al. 2011); and complex communities of tidepools (Hugget and Griffiths 1986; Metaxas and Scheibling 1993). On the other hand, intrinsic factors (region of origin and geographic range size) appeared to be major determinants of the non-randomness of the community of introduced birds (Blackburn and Cassey 2007), whereas the non-random structure of anuran assemblages depended on species-specific acoustic spectrum and vocalization patterns (Bourne and York 2001).

Parasites represent a convenient model for community ecology studies for the following reasons. Firstly, hosts are typically parasitized by several parasitic species that often share the same trophic level. Secondly, parasite communities are characterized by discrete boundaries, being fragmented

among host individuals (into infracommunities), host populations (into component communities), and host communities (into compound communities) (Holmes and Price 1986; Combes 2001; Poulin 2007b). Each host individual therefore harbours a replicate infracommunity of parasite species derived from the greater ensemble of parasite species that form the component or compound community (Bush et al. 1997). It is thus not surprising that patterns of community organization have been repeatedly studied in various parasite taxa exploiting various host taxa. These studies revealed non-random patterns of organization in some, albeit not other parasite communities (Worthen and Rohde 1996; Poulin 1997; Rohde et al. 1998; Gotelli and McCabe 2002; Krasnov et al. 2006b; Tello et al. 2008; Van der Mescht et al. 2016).

Similar to free-living species, parasite community structure may be influenced by both host-associated and parasite-associated factors. For example, host body size, gender, dietary specialization, geographic range size, and density have been found to affect the occurrence and manifestation of non-random patterns in various parasite communities (Poulin and Valtonen 2001; Calvete et al. 2004; Krasnov et al. 2005a, 2011). From the parasite perspective, community structure can be affected by, for example, parasite prevalence or mean intensity (Poulin and Valtonen 2002) and degree of host specificity of the most common species (Matějusová et al. 2000).

Among host-associated factors, social and spatial behaviour may have a profound effect on parasite community structure. Although it has been shown that these factors strongly affect the abundance of parasites (e.g., Altizer et al. 2003) and diversity of parasite communities (e.g., Bordes et al. 2007), their effect on parasite community structure has rarely been studied. Moreover, to the

best of our knowledge, studies that specifically addressed this question dealt either with parasites of aquatic hosts (Gonzales and Poulin 2005) or ectoparasites of terrestrial hosts (Krasnov et al. 2010a; Van der Mescht et al. 2016), whereas the effect of host sociality or spatial utilization on the structure of communities of endoparasites in terrestrial environments is largely unknown. Among-individual interactions of group-living social hosts may lead to higher rates of parasite exchange within but not among social groups thus facilitating the manifestation of parasite community structure within a locality (Taffs 1976; Anderson 2000; Carlberg and Lang 2004). In contrast, large home ranges and high mobility of solitary host species may result in higher rates of parasite exchange between all individuals inhabiting a locality and thus hinder structuring of parasite communities. Alternatively, hosts with large individual home ranges could harbour richer parasite infracommunities, which, in turn, could lead to a higher likelihood of nested pattern due to a higher probability of the occurrence of host-specific parasite species (Gao and Perry 2016).

Here we studied community organization of gastrointestinal helminths in two South African rodent hosts, *Rhabdomys dilectus* and *Rhabdomys pumilio*. Although these two species are closely related (du Toit et al. 2012), they differ in their social and spatial behaviour (Schradin 2005; Schradin and Pillay 2005a). *Rhabdomys dilectus* is strictly solitary and highly mobile, whereas *R. pumilio* lives in groups of 8-30 individuals and is territorially conservative. Moreover, inter-individual contacts in *R. pumilio* occur mainly within a social group but not between social groups, even those living in close vicinity (Schradin and Pillay 2005a). Comparison of organization of parasite communities of these two species may give important insights into the effect of host social and spatial behaviour on parasite community structure. This is because relatedness of these hosts determines their similarity in a variety of morphological, physiological and likely immunological characteristics

relevant for parasites except for a pronounced difference in the degree of their sociality and pattern of space use. Recently, Van der Mescht et al. (2016) demonstrated that the difference in structure of ectoparasite communities harboured by these two hosts results from their contrasting social/spatial behaviour. It remains unknown whether the same rules apply to the community structure of their endoparasites.

Before any attempt is made to investigate factors affecting non-randomness in community assembly, such non-randomness should be established. Consequently, the first two questions asked were (a) whether helminth communities of the two hosts are characterized by non-random patterns and (b) whether the occurrence or the degree of these patterns differs between social and solitary hosts. To answer these questions, we examined patterns of species co-occurrence in helminth infracommunities of *R. dilectus* and *R. pumilio* to test whether species composing these assemblages are associated, either positively (i.e., co-occur in the same host individuals more frequently than expected by chance) or negatively (i.e., co-occur less frequently than expected by chance). *Rhabdomys dilectus* and *R. pumilio* differ in both sociality and spatial (= territorial) behaviour, it is thus difficult to disentangle the effects of these traits on parasite community structure. However, differential effects of host sociality versus territorial behaviour could be inferred from between-host differences in a given pattern. For example, if an aggregative pattern of helminth co-occurrences is manifested more strongly in *R. pumilio* than in *R. dilectus*, then host social structure is likely the main driver of parasite community structure (e.g., Taffs 1976). If, however, helminth community structure is manifested more strongly in *R. dilectus*, then host mobility may also play a role. Subsequently, we searched for nested patterns in helminth communities of each host species at two hierarchical scales, namely among infracommunities

within a locality and among component communities across localities. Communities are nested if species in species-poor assemblages comprise non-random subsets of the species occurring in successively richer assemblages (Patterson and Atmar 1986). However, if species are always absent from communities richer than the most depauperate one in which they occur, then these communities are considered to be anti-nested (see Poulin and Guégan 2000). Although this pattern is rarely reported, it nevertheless indicates non-random assembly of species due to some unknown structuring forces (Poulin and Guégan 2000; Krasnov et al. 2005a). Recently, Almeida-Neto et al. (2007) suggested that ‘‘anti-nestedness’’ should not be designated as the opposite of nestedness but rather each “anti-nested” pattern should be considered as a distinct structural arrangement. We predicted that helminth infracommunities of *R. pumilio* would be more likely to display nested patterns than those of *R. dilectus* due to the group-living nature of the former. Within a locality, social *R. pumilio* live in aggregated groups with weak (if at all) between-group overlap in home ranges. This may promote frequent contacts within a group, but rare or no contacts between groups. In contrast, high mobility and/or large home ranges of solitary *R. dilectus* may result in high rates of both intraspecific and interspecific contacts making a nested pattern in helminth communities unlikely. Given that (a) parasite assemblages across host populations are predominantly affected by biogeographic (i.e., gains and losses of parasite species) rather than epidemiological (i.e., births and deaths of parasite individuals) processes (Brooks et al. 2006) and (b) biogeographic processes are the main drivers of nestedness via the dynamic of species gains and losses (Patterson and Atmar 1986), we expected that nestedness of component communities of helminths will be manifested (a) more strongly than that of their infracommunities and (b) similarly in both host species. The third question we asked was whether the occurrence or the degree of non-randomness is associated with the abundance of helminths, their prevalence and diversity of their assemblages and expected

that the manifestation of non-randomness will be stronger in host populations with higher levels of infection (Poulin and Guégan 2000; Poulin and Valtonen 2002).

Rhabdomys dilectus inhabits the mostly mesic eastern regions of South Africa where the main vegetation type is grass, whereas *R. pumilio* occurs in the more xeric western regions of South Africa. Environmental factors have been shown to substantially affect ectoparasite community structure (e.g., Van der Mescht et al. 2016). However, intestinal helminths occupy a habitat within the body of their host, which shields them from external environmental conditions. Although environmental factors may affect individual helminth species via free-living immature stages (e.g., Spickett et al. 2017 for *R. dilectus* and *R. pumilio*), the effect of these factors on helminth communities is likely much weaker than that on ectoparasites communities.

Methods

Mammal sampling and helminth recovery

Rodents were trapped at 20 localities across South Africa (see Appendix B, S. 2) using Sherman-type live traps, baited with a peanut butter-oats mixture. In each locality, traps were set out in 3-5 lines (50-100 m length) with 10 m distance between traps, checked twice daily and closed during the heat of the day (11:00-15:00) in summer and the cold nights (17:00-8:00) in winter. Adult animals (*R. pumilio* or *R. dilectus*) were targeted and placed in plastic bags and immediately euthanized by intraperitoneal injection with Sodium Pentobarbitone (200 mg/kg). All helminths, represented by cestodes and nematodes, were recovered from the gastrointestinal tract and stored in 70% ethanol. Identifications were based on relevant keys and descriptions of various authors. Voucher specimens of each species have been deposited in the National Collection of Animal

Helminths at the ARC-Onderstepoort Veterinary Institute, South Africa (accession number of nematodes: S/2016/38) and the Finnish Museum of Natural History, Luomus, Finland (accession numbers of cestodes: KN 3646-KN 3668). Data on sampling design, environmental conditions at the sampling localities, abundance and prevalence of helminth species as well as on helminth life cycles and transmission modes can be found elsewhere (Spickett et al. 2017).

Data analysis

Species co-occurrence

The data for each trapping session (a given locality sampled in a given season; separately for the two host species) were organized as a presence/absence matrix in which a row represented a helminth species and a column represented a host individual. We included in the analysis only trapping sessions in which at least three helminth species were recorded across all host individuals. To detect non-randomness of entire matrices, we applied null model analyses as implemented in the software EcoSim Professional 1.2 (Entsminger 2014). We chose to use the *C*-score (average number of checkerboard units that are found for each pair of species; Stone and Roberts 1990) as a metric of co-occurrence of helminth species because it has good Type I error properties (Gotelli 2000) and is one of the most commonly used metrics of community structure (Gotelli and McCabe 2002; Gotelli and Rohde 2002; Krasnov et al. 2006a; Gotelli and Ulrich 2010). An observed *C*-score was calculated for each presence/absence matrix and compared with the mean *C*-score calculated from the *C*-score of each of 5000 randomly assembled null matrices measuring the probability that the observed index was larger or smaller than expected by chance. A *C*-score larger than expected by chance [observed (O) > expected (E)] indicates negative co-occurrences (i.e.,

species are segregated), while a *C*-score smaller than expected by chance (O < E) indicates positive co-occurrences (i.e., species are aggregated) (Gotelli 2000).

A number of algorithms can be used to assemble simulated matrices. In all of them, species occurrences are randomized but the row sum (i.e., incidences of species) or column sum (i.e., number of species per site or, in our case, per host individual) can be either preserved (= fixed) or unconstrained (= equiprobable). For the sake of biological realism, we used an equiprobable-fixed algorithm. The rationale behind the unconstrained row sum was that this sum essentially reflected the number of host individuals that a helminth species was able to exploit. Given that all host individuals within a locality were conspecifics, we assumed that a helminth species could potentially exploit as many of them as possible. On the other hand, a host individual could be constrained in the number of helminth species that it is able to harbour and respond to multiple helminth challenges either by mounting strong immune response or merely dying (Folstad and Karter 1992; Arneberg 2002; Moore and Wilson 2002; Krasnov et al. 2005a, 2010a; Wirsing et al. 2007). Consequently, the column sums were preserved in the null matrices. Re-analysis of the data using an equiprobable-equiprobable algorithm provided essentially the same results (see Appendix B, S. 3).

For each locality matrix, we calculated the standardized effect size (SES). SES measures the number of standard deviations that the observed index (i.e., the *C*-score) is above or below the mean index of simulated matrices (Gotelli and McCabe 2002) and is calculated as the difference between the observed index and the mean of simulated indices divided by the standard deviation of simulated indices. Assuming a normal distribution of deviations, the null hypotheses are that

(a) the average SES across a set of presence/absence matrices equals zero and (b) 95% of the observations are expected to fall between 2.0 and -2.0. To test these null hypotheses for a set of locality matrices (separately for *R. dilectus* and *R. pumilio*), we used one-sample *t*-tests.

To test whether helminth community structure (measured via SES) differed between *R. dilectus* and *R. pumilio*, we applied linear mixed-effects models (LME) using package "nlme" (Pinheiro et al. 2016) for R 3.3.0 statistical environment (R Core Team 2016). Some localities were sampled more than once (in different seasons). Matrix size (number of rows × number of columns) may affect statistical power of the analysis and affect SES values (see Gotelli and McCabe 2002 for details). Consequently, we included sampling locality as a random factor and matrix size as an additional explanatory variable in the model. Least-squares means were calculated using package "lsmeans" for R (Lenth 2016).

To understand whether the degree of community structure was associated with abundance of helminths, their prevalence and diversity of their assemblages, we calculated the mean number of helminth individuals per infected host (mean intensity of infection), mean number of helminth species found per infected host (mean helminth species richness) and prevalence of infection by all helminth species for each sampling replicate. We ran LME (separately for *R. dilectus* and *R. pumilio*) with absolute values of SES as a response variable and mean intensity of infection, mean helminth species richness, prevalence of helminth infection and matrix size as explanatory variables (= fixed effects). Distribution of SES values did not significantly deviate from normality (Shapiro-Wilk $W = 0.92$ for *R. dilectus* and Shapiro-Wilk $W = 0.95$ for *R. pumilio*, $P > 0.37$ for both). In all models, sampling locality was included as a random factor (see above). After the

initial run of the model, we generated a set of models with subsets of terms in the global model using the function “dredge” of the R package “MuMIn” (Barton 2016) and selected the best model using Akaike’s Information Criterion (AIC) corrected for sample size. We then ran the best model.

We identified particular pairs of helminth species that co-occurred significantly, being either aggregated or segregated. This was done by calculating the *C*-score for each pair of species and by identifying its significance using methods proposed by Gotelli and Ulrich (2010). The most liberal method for identifying significance of the *C*-score is the confidence limit criterion (CL) when an observed metric for each pair is related to confidence limits of a simulated random distribution, so that pairs with scores outside the 95% confidence limits are considered significantly segregated or aggregated (if the observed *C*-score is greater or smaller than the mean of the simulated *C*-scores for a given pair, respectively). However, if the number of species pairs in a matrix is large, even 5% of them falling outside the 95% confidence limits merely by chance can represent a large absolute number (Gotelli and Ulrich 2010). To avoid this, the weakest 5-10% of significant pairs can be removed (Sfenthourakis et al. 2006; Gotelli and Ulrich 2010) or the significance level can be adjusted using the Benjamini and Yekutieli (BY) criterion (Benjamini and Yekutieli 2001; Gotelli and Ulrich 2010). Two additional conservative criteria based on an empirical Bayes approach that allow to identify significantly co-occurring pairs of species were introduced by Gotelli and Ulrich (2010) and are realized in the program PAIRS (Ulrich 2008). In these methods, instead of a comparison between observed and expected scores of each species pair, the observed frequency distribution of scores was compared with the frequency distribution of scores generated by the null model. The observed *C*-score for each species pair was calculated, rescaled to a range from 0 to 1 and grouped into a number of classes of evenly spaced bins (22

classes in Gotelli and Ulrich 2010). Subsequently, 1000 null matrices were assembled and mean and confidence limits of the expected number of species pairs within each bin were calculated from these null matrices. Species pairs within each bin were ordered according to their observed *C*-scores and pairs that fell above the mean (Bayes M criterion) or confidence interval (Bayes CL criterion) for the expected number of species were considered significant. The three conservative methods (BY, Bayes M and Bayes CL criteria) have been shown to reduce the false detection error rate and, consequently, are considered to be useful in analyses of community structure, although none of them was found to be ideal in reliable detection of non-random species pairs in both simulated and empirical matrices (see Gotelli and Ulrich 2010 for details).

Nestedness

We analysed nestedness among infracommunities (helminth assemblages in individual hosts) within a locality (IC analyses) and among component communities (helminth assemblages in all host individuals in a locality) within host species (CC analyses). Similar to analyses of co-occurrences, the data were organized as presence/absence matrices (separately for *R. dilectus* and *R. pumilio*) in which rows represented helminth species and columns represented individual hosts within a locality (IC analyses) or localities within a host species (CC analyses). IC analyses were carried out for samples (i.e., localities) in which no less than four helminth species were found, whereas all localities were included for CC analyses.

We estimated nestedness for each matrix. A variety of nestedness metrics has been developed such as the *C* metric (Wright and Reeves 1992), the matrix temperature *T* (Atmar and Patterson 1993), and the discrepancy measure *d* (Brualdi and Sanderson 1999). Each of these metrics has

advantages and disadvantages (see review in Almeida-Neto et al. 2008). The most important problem with these metrics is that they are prone to type I statistical errors, often detecting nested patterns erroneously (Almeida-Neto et al. 2008). Almeida-Neto et al. (2008) proposed a metric NODF (Nestedness metric based on Overlap and Decreasing Fill) that possesses better statistical properties. This metric is based on standardized differences in row and column fills and paired matching of occurrences. Importantly, NODF allows the calculation of nestedness independently among rows or columns, thus providing the possibility to evaluate nestedness only among sites or only among species. In addition, absolute values and Z-transformed scores of NODF appeared to be insensitive to matrix shape and size, although its Z-score under a fixed-fixed null model (but not under an equiprobable model) is somewhat sensitive to matrix fill when it is lower than 10% (Almeida-Neto et al. 2008). In our study, matrix fill ranged from 16% to 53%. Consequently, we used NODF and evaluated nestedness among columns (i.e., nestedness in species composition among individual hosts or localities; N_{COL}) using program ANINHADO (Guimaraes and Guimaraes 2006) (note that in the original presentation of ANINHADO and opposite to our data organization, columns represent species, while rows represent localities). For each matrix, we also calculated a SES as a Z-transformed score and compared the observed index to the distribution of indices calculated for 1000 randomly assembled null matrices measuring the probability that an observed index (O) is larger or smaller than expected by chance (E). $O > E$ indicates a nested pattern, whereas $O < E$ indicates significant anti-nestedness. Simulated matrices were assembled by Monte Carlo procedures. Similar to the analyses of co-occurrences, presences were randomly assigned within rows (i.e., among host individuals) but not within the columns (that is, within host individuals) of simulated matrices in IC analyses (see above). In CC analyses, presences were randomly assigned both within rows and within columns. To test the null hypotheses that SES

across localities within a host species equals zero, we used one-sample *t*-tests. We compared the degree of infracommunity nestedness between host species using *t*-tests. Multiple analyses requested adjustments of the alpha-level due to an inflated Type I error rate (e.g., Bonferroni correction). However, this approach has been criticized by statisticians and ecologists because it often leads to incorrect acceptance of the false null hypothesis (Perneger 1999; Moran 2003; García 2004). Nevertheless, we performed sequential Holm-Bonferroni corrections of the significance level using Holm-Bonferroni Sequential Correction: an Excel Calculator (Gaetano 2013) separately for *R. dilectus* and *R. pumilio* and present our results with and without these corrections. Similar to the analyses of species co-occurrence, we ran LME (separately for the two hosts) with absolute values of SES as a response variable; mean intensity of infection, mean helminth species richness and prevalence of helminth infection as explanatory variables (= fixed effects) and sampling locality as a random factor (see above). Similar to the co-occurrence analyses, distribution of SES values for nestedness did not significantly deviate from normality (Shapiro-Wilk $W = 0.96$ for *R. dilectus* and Shapiro-Wilk $W = 0.92$ for *R. pumilio*, $P > 0.34$ for both). Subsequently, we selected the best model using AIC and ran it. In addition, we tested whether helminth prevalence, intensity of infection and mean helminth species richness in *R. pumilio* differed between anti-nested and non-nested assemblages (see Results) (Poulin and Valtonen 2001) using *t*-tests.

Results

Overall, 778 individual rodents were captured from 20 localities across South Africa (292 *R. dilectus* and 486 *R. pumilio*). *Rhabdomys dilectus* harboured 19 nematode and 7 cestode species while *R. pumilio* harboured 10 nematode and 5 cestode species. Seven helminth species (4

nematodes and 3 cestodes), were shared between the two rodent species, however, they also harboured their own specific helminths (see Spickett et al. 2017). The most abundant and prevalent species harboured by *R. dilectus* was *Neoheligmonella capensis* while *Heligmonina spira* was the most abundant and prevalent species in *R. pumilio*.

Species co-occurrence

The *C*-score of observed presence/absence matrices differed significantly from those of simulated matrices in 9 of 12 populations of *R. dilectus* and 10 of 12 populations of *R. pumilio* (Table 4.1). In all cases when *C*-scores calculated for observed matrices differed significantly from those calculated for simulated matrices, their values were smaller than expected by chance. SES values for *C*-scores averaged within host species differed significantly from zero and from -2.0 in both *R. dilectus* (t = -4.16 and t = -4.93, respectively; $P < 0.05$ for both) and *R. pumilio* (t = -4.48 and t = -5.43, respectively; $P < 0.05$ for both). Average SES values across host populations did not differ between the two host species (coefficient value = 1.91 ± 2.19, $F = 0.49$, $P = 0.48$).

Selection of the best model explaining the absolute value of SES resulted in a model that included mean helminth species richness only for *R. dilectus* [corrected AIC = 84.5, AIC weight = 0.24; absSES = 0.29+6.02×(mean helminth species richness); $F = 8.15$, $P = 0.01$] and prevalence only for *R. pumilio* [corrected AIC = 78.7, AIC weight = 0.52; absSES = -11.87 + 25.21×(prevalence); $F = 5.58$, $P = 0.03$]. Model selection tables are presented in Appendix B, S. 4. In other words, and given mostly negative SES values, the strength of positive associations among species in helminth assemblages (measured via SES of the *C*-score) increased with an increase in the mean number of helminth species in *R. dilectus* and in prevalence of infection in *R. pumilio* (Fig. 4.1).

There was a total of 209 species pairs in 12 helminth assemblage matrices in *R. dilectus* (from 3 to 66 pairs per assemblage) and 93 species pairs in 12 helminth assemblage matrices in *R. pumilio* (from 3 to 66 pairs per assemblage). Of these, 22 pairs in seven assemblages of *R. dilectus* (16 unique pairs) and 14 pairs in eight assemblages of *R. pumilio* (eight unique pairs) were significantly associated by all criteria (including the most conservative BY criterion) (Table 4.2). The number of these pairs did not correlate with the total number of unique species pairs in an assemblage (Spearman rank order correlation coefficient = 0.54 for *R. dilectus* and Spearman rank order correlation coefficient = 0.68 for *R. pumilio*; $P > 0.05$ for both). All significantly associated pairs of helminth species in assemblages harboured by *R. dilectus* were positively associated (Table 4.2). In contrast, among 14 significantly associated species pairs in assemblages harboured by *R. pumilio*, there were six (five unique) negatively associated pairs and eight (three unique) positively associated pairs. Identities of significantly associated species pairs are presented in Table 4.3. Interestingly, one pair of species, *Heligmonina spira* and *Syphacia* sp., was found to be positively associated in both *R. dilectus* and *R. pumilio*. In contrast, *Neoheligmonella capensis* and *Syphacia* sp. appeared to be positively associated in assemblages harboured by *R. dilectus*, but negatively associated in assemblages harboured by *R. pumilio*.

Table 4.1 Observed (O) and expected by chance (E; mean C-score of 5000 simulated matrices) values of *C*-score for presence/absence matrices of helminth communities. SES – standardized effect size, *P*-value is related to the difference between observed and expected values of the *C*-score (O < E if SES is negative or O > E if SES is positive). See Appendix B, S. 2 for abbreviations of the locality names.

Host	Locality	*C*-score		SES	*P*
		O	E		
R. dilectus	CHR	3.48	12.15	-16.74	<0.001
	DFR	6.83	28.58	-9.66	<0.001
	ELN	7.60	31.97	-12.47	<0.001
	ELS	5.64	55.68	-25.94	<0.001
	TSC	2.95	41.67	-24.6	<0.001
	KRP1	28.00	51.34	-5.99	<0.001
	KRP2	15.30	66.68	-18.55	<0.001
	KRP3	5.66	30.22	-9.72	<0.001
	KRP4	1.33	0.67	1.41	0.11
	RVR	10.2	21.84	-8.35	<0.001
	NDR	0.80	1.34	-1.37	0.09
	SDR	3.00	2.81	0.44	0.41
R. pumilio	ABR	12.66	15.38	-2.62	0.02
	ATC	13.17	13.05	0.1	0.58
	BTS	0.00	20.23	-9.11	<0.001
	EBR	5.16	45.99	-14.73	<0.001
	HBR	19.00	26.15	-3.14	0.01
	HHR	10.83	74.13	-17.33	<0.001
	JHR	32.30	117.97	-21.85	<0.001
	JKL	54.33	51.40	0.84	0.16
	MBR1	29.50	71.71	-13.59	<0.001
	MBR2	16.10	36.61	-9.06	<0.001
	MBR3	12.87	45.83	-15.31	<0.001
	MBR4	7.90	19.03	-7.21	<0.001

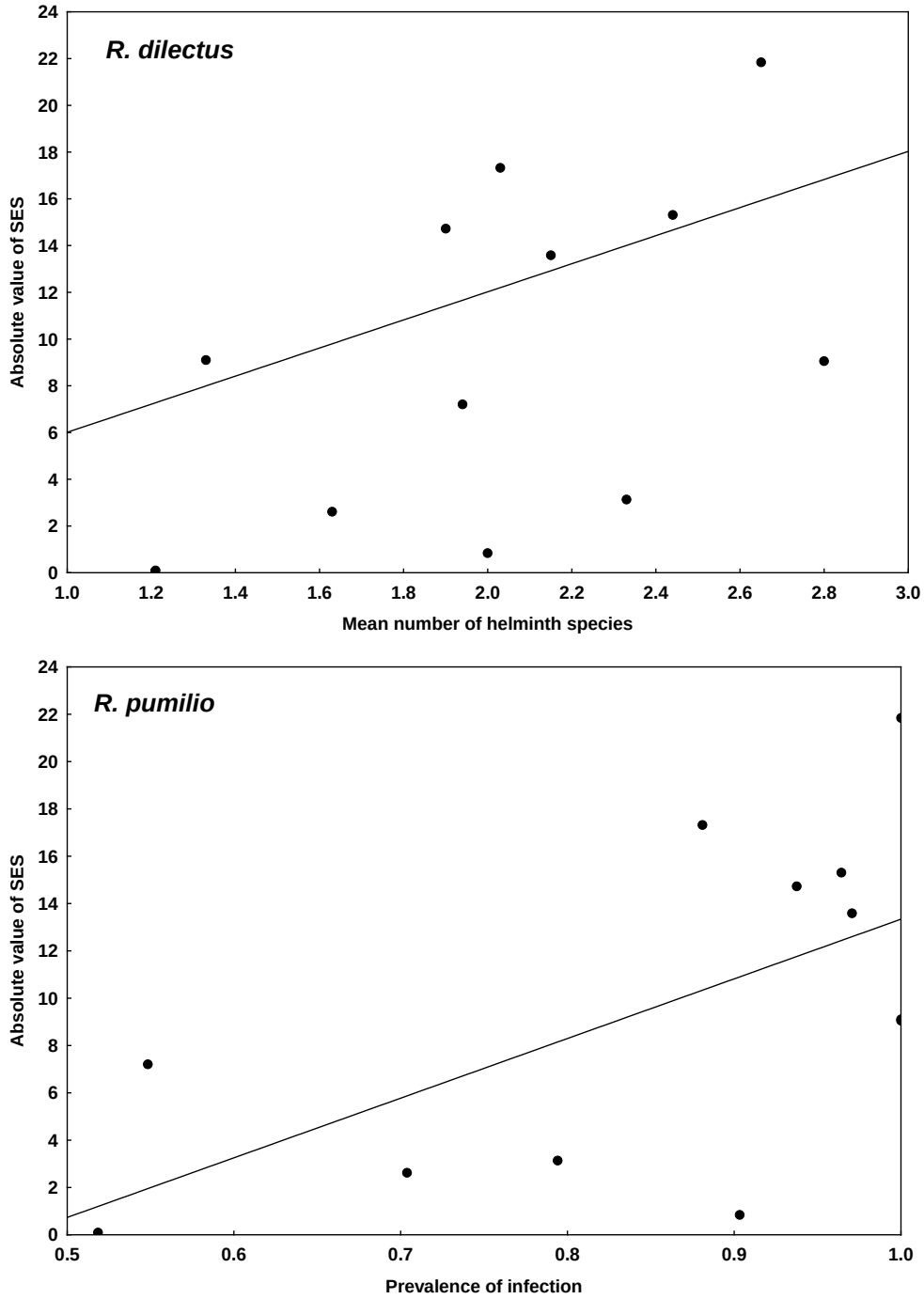

Fig. 4.1 Relationships between manifestation of helminth co-occurrences (measured as SES of the C-score) and mean helminth species richness (*Rhabdomys dilectus*) or prevalence of infection (*R. pumilio*).

Table 4.2 Species pairs in helminth assemblages that were identified as significantly associated by CL and BY criteria (see text for explanations). Only assemblages in which at least one species pair was identified by at least one of the criteria as significant are presented. CL – simple confidence limits criterion, BY criterion – after sequential Bonferroni correction (Benjamini and Yekutieli 2001). TP/PA/NA - Total number of pairs/number of significantly positively associated pairs/number of significantly negatively associated pairs ($P<0.05$ for 3 pairs, $P<0.01$ for 6 pairs and $P<0.001$ for 27 pairs). Z (CL) - Z-score case for species with observed scores greater or smaller than the upper or lower confidence limit for that pair; Z(BY) - false error rate corrected Z-scores according to the method of Benjamini and Yekutieli (2001). Z_{CL} and Z_{BYf} above slash (/) are for positively associated pairs and below slash for negatively associated pairs. See Appendix B, S. 2 for abbreviations of the locality names

Host	Locality	TP/PA/NA	Z_{CL}	Z_{BYf}
R. dilectus	CHR	66/2/-	-6.66 – -5.32/-	-6.66 – -4.98/-
	DFR	6/1/-	-4.11/-	-3.91/-
	ELN	10/2/-	-3.77 – -3.29/-	-3.91 – -2.99/-
	ELS	28/5/-	-7.73 – -2.61/-	-7.73 – -2.10/-
	KPR3	10/1/-	-5.52/-	-5.52/-
	RVR	10/1/-	-2.87/-	-2.52/-
	TCS	45/10/-	-5.11 – -2.72/-	-4.83 – -2.19/-
R. pumilio	EBR	6/-/1	-/3.81	-/3.59
	HHR	6/1/1	-3.26/6.18	-3.01/6.18
	JHR	6/2/-	-4.80 – -2.82/-	-4.58 – -2.47/-
	JKL	3/1/-	-4.79/-	-4.57/-
	MBR1	10/-/1	-/5.63	-/5.63
	MBR2	10/1/1	-3.21/3.15	-2.90/2.83
	MBR3	15/2/2	-4.52 – -3.27/3.36 - 3.96	-4.26 – -2.92/3.02 – 3.66
	MBR4	10/1/-	-3.00/-	-2.66/-

Table 4.3 Identities of helminth species in significantly associated species pairs in the assemblages harboured by *Rhabdomys dilectus* and *R. pumilio*. N – number of assemblages in which a given pair was found to be significantly associated.

Host	Association	Species 1	Species 2	N
R. dilectus	Positive	*Neoheligmonella capensis*	*Syphacia* sp.	3
		Neoheligmonella sp.	*Syphacia* sp.	3
		Heligmonina spira	*Neoheligmonella* sp.	2
		Heligmonina spira	*Syphacia* sp.	2
		Heligmonina spira	*Paralibyostrongylus* sp.	1
		Heligmonina spira	*Rodentolepis* sp.	1
		Heligmonina spira	*Trichostrongylus* sp.	1
		Neoheligmonella capensis	*Heligmonina boomkeri*	1
		Neoheligmonella sp.	*Inermicapsifer* sp.	1
		Neoheligmonella sp.	*Paralibyostrongylus* sp.	1
		Neoheligmonella sp.	*Rodentolepis* sp.	1
		Neoheligmonella sp.	*Trichostrongylus* sp.	1
		Raillietina trapezoides	*Meggittina baeri*	1
		Syphacia sp.	*Paralibyostrongylus* sp.	1
		Trichostrongylus sp.	*Paralibyostrongylus* sp.	1
		Trichostrongylus sp.	*Syphacia* sp.	1
R. pumilio	Positive	*Heligmonina spira*	*Neoheligmonella capensis*	6
		Heligmonina spira	*Paralibyostrongylus* sp.1	1
		Heligmonina spira	*Syphacia* sp.	1
	Negative	*Paralibyostrongylus* sp.1	*Neoheligmonella* sp. 1	1
		Syphacia sp.	*Neoheligmonella capensis*	1
		Syphacia sp.	*Neoheligmonella* sp. 1	1
		Syphacia sp.	*Paralibyostrongylus* sp.1	1
		Syphacia sp.	*Trichostrongylus probolurus*	1

Nestedness

The degree of nestedness in species composition of helminth infra- and component communities harboured by *R. dilectus* and *R. pumilio* is presented in Table 4.4. SES of the infracommunity nestedness across localities did not differ significantly from zero in either *R. dilectus* or *R. pumilio* (t = 0.83 and t = -1.48, $P > 0.17$ for both). Nevertheless, helminth infracommunities in *R. dilectus* in one locality demonstrated a tendency to be nested (CHR) and in another locality to be anti-nested (ELN, Table 4.4). However, these trends appeared to be non-significant after sequential Holm-Bonferroni adjustment of the alpha-level. In *R. pumilio*, helminth infracommunities in two of 10 localities appeared to be significantly nested and in five of 10 localities significantly anti-nested (Table 4.4). Significance of anti-nestedness of helminth infracommunities in all latter localities did not change after Holm-Bonferroni adjustment of the alpha-level.

The best models of the absolute values of SES (Z-scores) included intercept only for both species (AIC weight = 0.50 and AIC weight = 0.52 for *R. dilectus* and *R. pumilio*, respectively). Model selection tables are presented in Appendix B, S. 5. This suggested that the probability of helminth infracommunities to be structured in terms of nestedness was not associated with the level of infection and not affected by the diversity of helminths. Nevertheless, intensity of infection in *R. pumilio* (but not prevalence or helminth species richness) was marginally significantly lower in localities with anti-nested assemblages than in other localities (t = -2.29, P = 0.05 for intensity of infection; t = -1.37, P = 0.21 for prevalence and t = -1.75, P = 0.12 for species richness; Fig. 4.2).

Component communities of helminths infecting *R. dilectus* demonstrated significant nested patterns (Table 4.4). In contrast, communities harboured by *R. pumilio* were not nested (Table 4.4).

Table 4.4 Summary of results of nestedness analysis for infra- and component helminth communities of *Rhabdomys dilectus* and *R. pumilio*. N_{COL} – nestedness among infra-(IC) or component (CC) helminth communities, SES – standardized effect size (Z-score), *P* (O > E) and *P* (O < E) – probabilities that observed metric is larger or smaller than mean of 1000 simulated matrices. * - significant after sequential Holm-Bonferroni adjustment of alpha level (see text for explanations). See Appendix B, S. 2 for abbreviations of the locality names.

Host	Scale	Locality	N_{COL}	SES	*P* (O > E)	*P* (O < E)
R. dilectus	IC	CHR	41.47	1.72	0.04	
		DFR	27.37	0.15	0.44	
		ELN	17.14	-2.04		0.02
		ELS	38.38	1.00	0.14	
		KPR2	20.87	-1.02		0.15
		NDR	46.67	1.32	0.09	
		RVR	22.63	0.72	0.24	
		SDR	7.14	-0.05		0.48
		TCS	41.81	1.26	0.1	
	CC		20.60	2.70	0.003*	
R. pumilio	IC	ABR	0.00	-3.27		0.0005*
		ATC	0.00	-2.50		0.006*
		EBR	31.72	0.05	0.48	
		HBR	0.00	-5.88		<0.0001*
		HHR	0.00	-5.88		<0.0001*
		JHR	43.80	2.33	0.01	
		MBR1	0.00	-5.02		<0.0001*
		MBR2	39.78	1.20	0.11	
		MBR3	40.55	1.38	0.08	
		MBR4	40.20	1.99	0.02	
	CC		13.35	0.19	0.42	

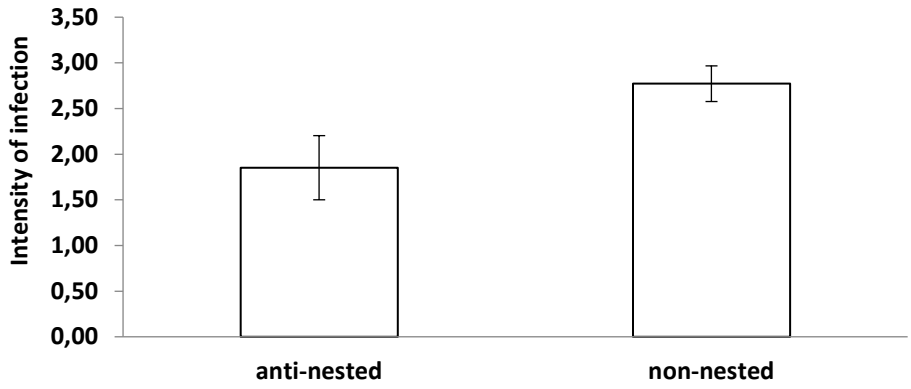

Fig. 4.2 Intensity of infection by helminths (log-transformed) in *Rhabdomys pumilio* in localities with anti-nested and non-nested helminth assemblages.

Discussion

The results of this study supported some but not all of our predictions regarding the effect of host social and spatial behaviour on the manifestation of helminth community structure. First, the general pattern of helminth co-occurrences was similar in the two hosts, although helminth infracommunities of *R. dilectus* and *R. pumilio* differed in the relative frequency of positive and negative pairwise species co-occurrences. Second, nestedness-related patterns in helminth infracommunities were found in *R. pumilio* but not *R. dilectus*, whereas component communities of *R. dilectus* but not *R. pumilio* were significantly nested. Third, although the level of infection was generally associated with the manifestation of non-randomness in helminth assemblages, different facets of infection affected different structure patterns in the two hosts. In general, our results did not allow us to distinguish between the effects of host sociality and spatial behaviour on community structure of intestinal helminths, but rather demonstrated the effect of the combination of these traits.

Species co-occurrence

In general, helminth infracommunities of both *Rhabdomys* species demonstrated aggregative rather than segregative structure. In other words, different helminth species co-occurred in the gastrointestinal tract of a host individual more frequently than expected by chance. This finding is in sharp contrast to the pattern found for intestinal helminths of fish (Dezfuli et al. 2001) and ungulates (Fellis et al. 2003) as well as ectoparasites of fish (Gotelli and Rohde 2002), but is similar to that reported for intestinal helminths of birds (Bush and Holmes 1986), shrews (Haukisalmi and Henttonen 1998) and arthropod ectoparasites of bats and rodents (Tello 2008; Krasnov et al. 2010b; Presley 2007, 2011), although inconsistent patterns of helminth co-occurrences were found in voles (Haukisalmi and Henttonen 1993). This suggests that patterns of parasite species co-occurrences are manifested differently in different host-parasite associations and may thus depend on both host and parasite species identity.

Positive co-occurrences of parasite species have often been described as a phenomenon of apparent or indirect facilitation (Levine 1999) mediated via the host (e.g., Krasnov et al. 2010b; Ulrich and Schmid-Hempel 2012). This mediation may act via two main mechanisms. First, some host individuals are preferred by any parasite species over other individuals because they encounter parasites more frequently or, for some reasons, their susceptibility is higher and/or immune defenses are weaker (e.g., Johnson and Hoverman 2014). Second, multiple parasite challenges may "overload" the immunological response of a given host either mechanically (Holmstad et al. 2008; Morrill and Forbes 2016) or as a result of a host's strategy to lessen energy expenditure for anti-parasitic defenses in favour of other traits (Jokela et al. 2000), making this host a favourable target for any parasite. Third, parasite-induced immunosuppression may produce positive overall

association among helminth species. The immunomodulatory influence of parasites on their host's and resulting effects on concurrent infections are well demonstrated (see e.g., Christensen et al. 1987; Behnke et al. 2001; Cox 2001). In rodents, some helminths, particularly nematodes, may be immunosuppressive, leading to higher susceptibility of the host not only to the given species, but also to various other species (Behnke et al. 1978, 2001; Rzepecka et al. 2006). In addition, some host individuals may receive multiple parasite species in one "package" due to an aggregated distribution of their infective stages. For example, many helminths could be simultaneously transmitted via ingestion of eggs through coprophagy, retrofection or grooming (Taffs 1976; Anderson 2000; Carlberg and Lang 2004). Similarly, co-occurrence of indirectly transmitted helminths could be simply due to both parasite species using the same intermediate host. Furthermore, positive co-occurrences might be associated with differential preferences of parasite species within a host. For example, *Syphacia* sp. co-occurred with five other helminth species. This parasite is usually found in the caecum of a host, whereas other helminths predominantly occupy the small intestine or stomach. Consequently, direct antagonistic interactions between *Syphacia* sp. and other helminths are unlikely.

Furthermore, the generally aggregative structure of helminth infracommunities in *R. dilectus* likely resulted from positive pairwise co-occurrences. However, there were more negatively than positively associated helminth pairs in the infracommunities of *R. pumilio*. Existence of both positive and negative associations between parasite species has earlier been reported for helminths in a bird host (Forbes et al. 1999). Obviously, negative associations between species may indicate certain antagonistic interactions such as competition (e.g., Lello et al. 2004). The occurrence of both positive and negative pairwise associations in *R. pumilio* suggests that both facilitation and

competition may act within the same community either together or when the intensity of each process varies spatially or temporally (Callaway and Walker 1997; Levine 1999). Moreover, both direct (among parasites) and indirect (between parasites and a host) effects should be considered when the general pattern of interactions in the context of an entire parasite community is of interest (Stone and Roberts 1991). Indeed, models of diffuse competition (competition in multi-species communities; Moen 1989) that took into account indirect interactions have concluded that a high number of species could not only reduce the intensity of interactions but could even lead to facilitation (Vandermeer 1990; Stone and Roberts 1991). Consequently, from a more synthetic view on a given community, species interactions may be considered as complex combinations of negative and positive processes (Levine 1999). In other words, multiple pairwise competitive interactions coupled with multiple pairwise facilitating interactions can result in a net positive general co-occurrence pattern when interactions with a shared competitor (host) are incorporated (Levine 1999).

Our results did not provide a straightforward answer regarding the effect of host social or spatial behaviour on helminth co-occurrence. On the one hand, only positive pairwise co-occurrence in *R. dilectus* and both positive and negative co-occurrence in *R. pumilio* hint at a stronger effect of host mobility when compared to the level of contact between hosts. Indeed, higher mobility of *R. dilectus* likely results in higher probability to encounter intermediate hosts of helminths or food contaminated with their eggs. On the other hand, home ranges of individual *R. pumilio* belonging to the same social group are lumped together, whereas those from different social groups occurring in the same locality practically do not overlap (Schradin and Pillay 2005a). Consequently, the rate of between-individual contact, albeit high over short distances (within a group), is almost zero

over longer distances (between groups) resulting in fragmented spatial distribution of host individuals. In a certain locality, a parasite species A can thus be consistently present in animals of a social group A, but consistently absent from animals of social group B. If the opposite is the case for a parasite species B, a negative pairwise association between these two parasites may arise. An intriguing case of *Neoheligmonella capensis* and *Syphacia* sp. positively co-occurring in *R. dilectus* and negatively co-occurring in *R. pumilio* suggests some kind of context-dependency of pairwise co-occurrences and warrants further investigation.

Nestedness

Recently, nested subset patterns were studied in flea communities of the same two hosts as in this study (Van der Mescht et al. 2016). Flea infracommunities of *R. pumilio* were generally nested, whereas this was not the case for *R. dilectus*. In the present study, we also found better manifestation of structure in *R. pumilio* than in *R. dilectus*, although helminth infracommunities of the former were more frequently anti-nested rather than nested. The reasons behind parasite community structure to be pronounced better in social than in solitary hosts are likely similar for both parasite groups and are associated with fragmented spatial distribution of social groups of the former (see Introduction). This fragmentation may be a cause of certain dynamics of losses and gains of parasite species manifested in a pronounced community structure such as anti-nestedness (Almeida-Neto et al. 2008). Solitary *R. dilectus* are usually highly mobile and their spatial distribution across large areas seems to be homogenous (du Toit et al. 2012). This may lead to frequent exchanges and replacements of helminth species between individual animals, thus making manifestation of nested or anti-nested patterns unlikely because, under such conditions, parasite species do not co-occur more or less frequently than expected by chance.

However, the opposite pattern was found in component communities of the two hosts with those of the solitary *R. dilectus* but not the social *R. pumilio* being nested. This suggests that scale-dependence of parasite community structure (e.g., Timi and Poulin 2003a; Krasnov et al. 2011) may be governed by host biology. Interestingly, component communities of fleas of these two hosts did not demonstrate any nested subset patterns whatsoever (Van der Mescht et al. 2016). This may support the idea of Patterson et al. (2009) that nested patterns in parasite communities are more probable for static and long-term infections by helminths than for short-term and dynamic infestations by ectoparasites.

Contrasting results about pattern (nestedness versus anti-nestedness) and the degree of manifestation of community structure between fleas (Van der Mescht et al. 2016) and helminths (this study) harboured by the same hosts, suggest that communities of these two groups of parasites are governed by different rules. This may be associated with differential strategies of transmission of fleas and helminths. Fleas are mainly transmitted via contacts with burrows belonging to other con- or heterospecifics (because pre-imaginal fleas develop largely off-host). Most helminths in this study are monoxenous and are ingested via contaminated food although some nematodes and all cestodes depend on arthropods as intermediate hosts. As a result, processes of gains and losses of fleas may differ from those of helminths at both infra- and component community scales.

We found anti-nested patterns of helminth infracommunities in some populations of *R. pumilio*, whereas in other populations patterns were nested. A continuum from nestedness to anti-nestedness was earlier reported for parasite infracommunities of fish (Poulin and Guégan 2000; Poulin and Valtonen 2001), bird (Calvete et al. 2004) and mammal hosts (Goüy de Bellocq et al.

2003). Poulin and Guégan (2000) suggested that this continuum could be considered in the framework of species coexistence models (Morand et al. 1999) and envisaged as a continuum of spatial organization of parasites from coexistence to competitive exclusion (but see Poulin and Valtonen 2001). In nested assemblages, parasites supposedly have reduced levels of interspecific aggregation relative to intraspecific aggregation and thus species coexistence is facilitated, whereas parasites occurring in anti-nested assemblages tend to show a strong interspecific aggregation resulting in species exclusion (Morand et al. 1999; Krasnov et al. 2005b). Our findings of many negatively-associated species pairs in helminth infracommunities of *R. pumilio* but not those of *R. dilectus*, coupled with the fact that anti-nestedness was revealed for the former but not for the latter, support this explanation. Mechanisms behind anti-nested pattern are largely unknown (Poulin and Guégan 2000; Almeida-Neto et al. 2007). Nevertheless, anti-nested pattern in island biogeography has been explained by the increasing likelihood of species endemism in large islands (Gao and Perry 2016). In application of this idea to parasite communities, anti-nestedness could occur in large infracommunities because of a higher probability of the occurrence of specialist species (Vazquez et al. 2005). However, this explanation for our case is weakened by the fact that mean helminth species richness did not differ between the two host species (Spickett et al. 2017).

Co-occurrence, nestedness and level of infection

Ulrich et al. (2009) stated that nestedness and aggregative patterns of species co-occurrences are interrelated because “a perfectly nested matrix is also a matrix with a maximum number of perfect pair wise species aggregations” (Ulrich et al. 2009, p. 13). Our results of the predominant anti-nestedness and negative pairwise co-occurrences in helminth infracommunities of *R. pumilio* provide some support that the opposite can also hold true despite the notion of Ulrich et al. (2009).

We found that strength of species co-occurrence correlated with helminth diversity in *R. dilectus* and with prevalence in *R. pumilio*. In addition, intensity of infection was lower in anti-nested than in non-nested assemblages. On the one hand, these results support the findings of earlier studies (Rohde et al. 1998; Poulin and Guégan 2000; Poulin and Valtonen 2001; Timi and Poulin 2003a; Gonzalez and Poulin 2005) that the probability of finding structure in parasite communities increases with prevalence, intensity and/or species richness of these communities. On the other hand, differential effects of these variables on (a) different structural patterns of helminth assemblages and (b) in different hosts suggest that community structure of parasites results from complex interactions between parasite-associated- and host-associated factors. The present study, while aiming to better appreciate the role of host sociality and spatial behaviour in shaping parasite community structure, was, however, limited to only two host species. Further investigation, by inclusion of additional host species with relevance to these host factors, could further clarify their role in parasite community structures. Furthermore, our current knowledge does not allow us to distinguish between cryptic helminth species, potentially present in our study. However, we believe that even if some species in our study represent a complex of cryptic species, this would form a minority in any community assemblage and, as a result, would not generally change our results (Dobson et al. 2008; Poulin 2014).

Chapter 5

Intra- and interspecific similarity in species composition of helminth communities in two closely related rodents from South Africa

Parasitology 144:1211-1220

Introduction

One of the main focus areas of modern ecological parasitology is to elucidate factors responsible for spatial variation in parasite community composition. Parasites ultimately depend on their hosts not only as sources of food but also as habitats in which they live and mate and as means of dispersal. It is thus not surprising that species composition of parasite communities is often shaped by host characteristics such as social structure (e.g., Bordes et al. 2007), age (Timi et al. 2010) or gender (Bordes et al. 2012). The off-host environment is another important factor affecting community composition in both ecto- (Krasnov et al. 1998) and endoparasites (Bush et al. 1990; Marcogliese and Cone 1991; Palmeirim et al. 2014). In other words, both host-associated and environment-associated factors are the reasons behind spatial variation in parasite community composition. While the reasons for environment-related variation in ectoparasite species composition are often clear (direct effects of abiotic factors; e.g., Krasnov et al. 2002), these reasons are less obvious for endoparasites such as gastrointestinal helminths. Nevertheless, whatever the ultimate factors affecting endoparasite community composition are, their actions likely result in at least two proximate (not mutually exclusive) causes shaping these communities. First, an absence or a presence of a given parasite in a community may be associated with the absence or presence of a suitable intermediate host in a given locality (Simkova et al. 2003). Second, environmental conditions may influence survival of free-living stages of some helminths (Hudson et al. 2006). Consequently, hosts living under different environmental conditions may differ in species composition of their parasite assemblages.

Spatial variation in parasite community composition has been repeatedly studied among and within host species (e.g., Bush 1990; Carney and Dick 2000; Poulin 2001, 2003; Timi and Poulin 2003b;

Karvonen et al. 2005; Krasnov et al. 2005d; Karvonen and Valtonen 2009; Froeschke et al. 2010). These studies revealed a plethora of factors underlying this variation with different factors acting in different host-parasite systems. This suggests the necessity of further studies to distinguish between general and system-specific factors responsible for spatial variation in parasite community composition (Karvonen and Valtonen 2009).

Among the reasons behind our still limited ability to understand general rules of spatial variation in species composition of parasite communities, the most striking are as follows. First, the majority of studies considered parasite assemblages in aquatic hosts, whereas parasites (especially, endoparasites) of terrestrial hosts have received less attention (but see Calvete et al. 2004; Froeschke et al. 2010; Palmeirim et al. 2014). Second, studies that compared patterns of spatial variation in parasite community composition among different hosts usually involved distantly related host species (e.g., Poulin 2003) that differ in multiple traits. As a result, it is often more difficult to reveal the most important host-associated factor(s) affecting spatial variation of parasite assemblages than when comparing closely related species. On the other hand, comparison of spatial variation of parasite communities in closely related allopatric species may allow a better understanding of the effect of environment on this variation because these species are likely similar in their morphological, physiological and immunological traits, but inhabit different environments.

Here, we studied patterns of spatial variation in species composition of the communities of gastrointestinal helminths harboured by two rodent hosts from South Africa, *Rhabdomys pumilio* and *Rhabdomys dilectus*. These two species are very closely related (du Toit et al. 2012) and until recently have even been thought to belong to the same species (e.g., Schradin 2005). Despite their

close relatedness, *R. pumilio* and *R. dilectus* differ sharply in their social and spatial behaviour. *Rhabdomys pumilio* is social and lives in groups comprising 8-30 adult individuals (Schradin and Pillay 2005a). This species inhabits the mostly xeric western region of South Africa (Fig. 5.1), with average rainfall varying from the more xeric central and northern parts (< 250 mm per annum; the greater Karoo region) to the less dry southern part (250-600 mm per annum; the Fynbos) (Mucina and Rutherford 2006). In contrast, *R. dilectus* is solitary and occurs in the mostly mesic eastern regions of South Africa in predominantly grassland habitats (Fig. 5.1). Furthermore, *R. pumilio* is characterized by high population densities (up to 171 individuals per hectare; Schradin and Pillay 2005b) and, consequently, small home ranges (ca. ± 250 m^2; Schradin 2005). In contrast, *R. dilectus* demonstrates low population densities (from 10 to 40 individuals per hectare; Perrin et al. 2001), follows a roaming strategy and therefore has much larger home ranges (± 2000 m^2; Ostfeld 1990; Schradin and Pillay 2004, 2005b; Schradin et al. 2010).

In this study, we focused on patterns of similarity in helminth species composition between and within these two host species because investigation of these patterns is one of the ways to understand the mechanisms behind spatial variation of parasite communities. We considered helminth assemblages of the two hosts at two hierarchical scales, namely infracommunities (assemblages of helminths in individual hosts) and component communities (assemblages of helminths in host populations; i.e. localities). Firstly, we asked whether the two hosts harbour similar helminth assemblages and whether these assemblages are more dissimilar between than within hosts. Secondly, we asked whether host social or spatial behaviour affects similarity in helminth species composition of infracommunities within as compared with that among localities. Higher within-locality than among-localities similarities presumably result from a higher

probability of helminth transmission among individual rodents. Consequently, we predicted that the effect of social behaviour will be manifested in the difference between within- and among-localities similarities in the infracommunity species composition being greater in social *R. pumilio* than in solitary *R. dilectus*. Alternatively, the effect of spatial behaviour will be manifested in this difference being higher in more mobile *R. dilectus* than in less mobile *R. pumilio*. Moreover, the difference between within- and among-localities similarities in helminth species composition could be associated with environmental conditions rather than with host traits. In particular, a more mesic environment may present favourable conditions for increased survival and transmission rates of free-living helminth stages, as well as for higher diversity and abundance of arthropods that serve as intermediate hosts for many helminths (e.g., Hudson et al. 2006; Dybing et al. 2013). If this effect of environment is strong, then the difference between within- and among-localities similarities is expected to be greater in the predominantly xeric *R. pumilio* than in the predominantly mesic *R. dilectus*. We further examined the effect of environmental conditions on patterns of similarity of helminth communities within *R. pumilio*. As mentioned above, the geographic range of this rodent in South Africa includes both the relatively more xeric greater Karoo region and the relatively less xeric Fynbos. If the environment incurs substantial effect on the pattern of similarity, then helminth infracommunities will be less similar between biomes than both among and within localities.

Finally, we asked whether similarity in species composition of helminth component communities decreases with an increase of geographic distance between host populations and, if yes, whether host spatial behaviour affects the rate of this decrease (Poulin 2003). A pattern of distance decay of similarity in species composition (a decrease of similarity between a pair of communities with

an increase of geographic distance between them; Nekola and White 1999) has been repeatedly reported for both ecto- (Krasnov et al. 2005d) and endoparasites (e.g., Poulin 2003). We predicted that the rate of distance decay of similarity will be higher in more territorially conservative *R. pumilio* than more mobile *R. dilectus*. Recently, Froeschke et al. (2010) reported the pattern of distance decay of similarity in species composition of helminth communities in *R. pumilio*. However, they evaluated pairwise similarity using the qualitative Jaccard index, whereas here we used the Bray-Curtis similarity index (Bray and Curtis 1957) that takes relative abundances of helminth species into account.

Materials and methods

Host sampling and helminth collection

Rodents were trapped at 20 localities across South Africa (Fig. 5.1) using Sherman-type live traps, baited with a peanut butter - oats mixture. Traps were set out in 3-5 lines (50-100 m length) with 10 m distance between traps, checked twice daily and closed during the heat of the day (11:00-15:00) in summer, and the cold nights (17:00-08:00) in winter. Adult animals (*R. pumilio* or *R. dilectus*) were placed in plastic bags and immediately euthanized by intraperitoneal injection with Sodium Pentobarbitone (200 mg/kg). All helminths were recovered from the gastrointestinal tract and stored in 70% alcohol. Identifications were based on relevant keys and descriptions of various authors. Abundance data were also recorded for each helminth species per host individual.

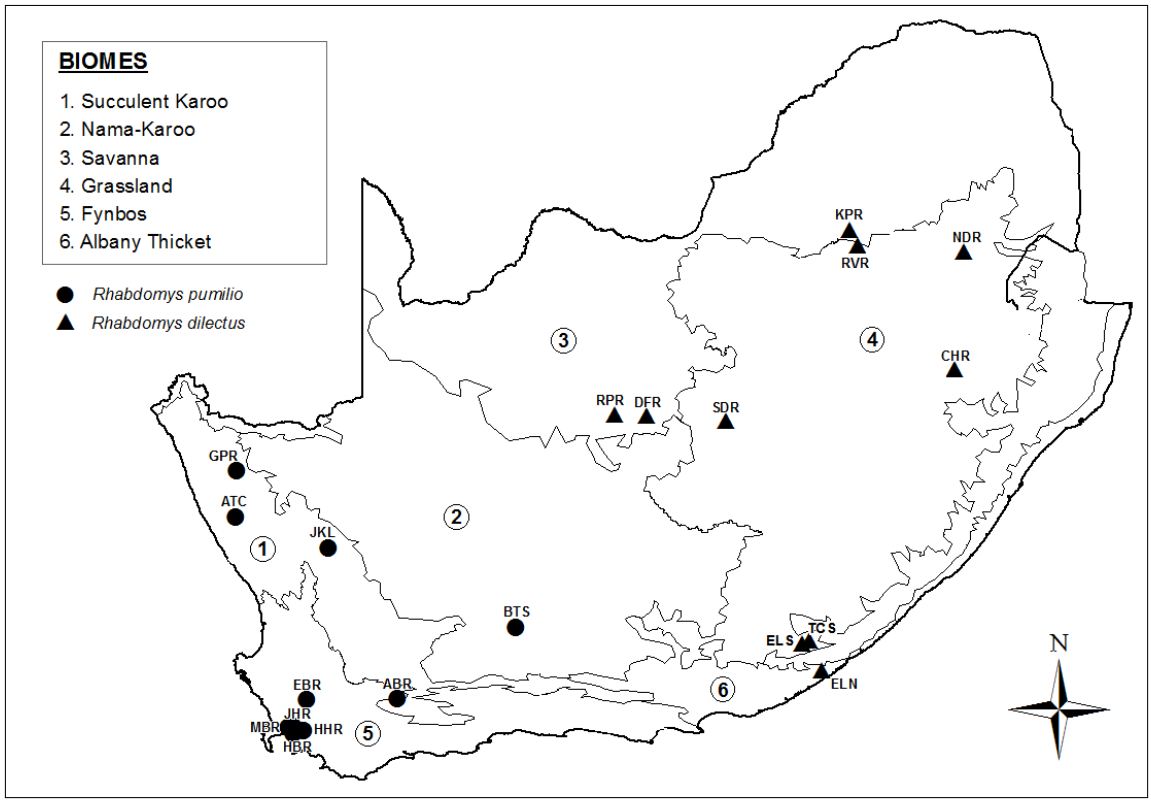

Fig. 5.1 Biome regions of South Africa, according to Mucina and Rutherford (2006) with sampling localities Locality names and abbreviations names are: Carolina (NDR); Pretoria site 1 (KPR); Pretoria site 2 (RVR); Springbok (GPR); Kimberley site 1 (DFR); Kimberley site 2 (RPR); Bloemfontein (SDR); Newcastle (CHR); Matjiesfontein (ABR); Stellenbosch site 3 (MBR); Stutterheim site 1 (ELS); Stutterheim site 2 (TCS); Beaufort West (BTS); Loeriesfontein (JKL); Garies (ATC); Somerset West (HBR); Stellenbosch site 2 (JHR); Stellenbosch site 1 (HHR); Wellington (EBR); East London (ELN).

Data analyses

To compare similarity in helminth species composition (a) between the two host species, (b) among localities within host species and (c) between biomes for *R. pumilio*, we carried out analyses of similarities (ANOSIMs) (Clarke and Green 1988; Clarke and Warwick 2001). ANOSIM operates on a matrix of similarities between samples. Consequently, prior to these analyses, the matrices of the Bray-Curtis similarities (Bray and Curtis 1957) in helminth species composition

between each pair of samples (i.e., individual hosts or localities) were constructed. The null hypothesis in ANOSIM is that there are no assemblage differences between groups of samples, i.e. among host individuals within a locality or among localities. The ANOSIM test statistic, *R*, usually varies between 0 and 1 (although theoretically it may vary from -1 to 1), where $R = 1$ indicates that all replicates within a group (i.e., host species or host population in a given locality) are more similar to each other than any replicates from different groups, whereas $R \approx 0$ suggests that similarities between and within groups on average are the same. Each analysis was based on 999 permutations and carried out using the routine ANOSIM implemented in the program Primer-7 (Clarke and Gorley 2015). Prior to analyses, the data entries (number of helminths of a given species in each host individual or mean abundance of a given helminth species in each locality) were log+1-transformed and standardized within a sample (i.e., a host individual or a locality) by total abundance. To visualize dissimilarity in species composition of helminth infracommunities, we applied multidimensional non-parametric scaling with a matrix of pairwise between-localities Bray-Curtis similarities in helminth species composition as input data. Then, we identified those helminth species that primarily accounted for differences between helminth infracommunities of the two hosts, each pair of localities for each host separately or between helminth assemblages of the Fynbos versus the greater Karoo region for *R. pumilio* (Clarke and Gorley 2015). This was carried out by decomposition of the Bray-Curtis similarity into contribution of each species using the routine SIMPER implemented in the program Primer-7 (Clarke and Gorley 2015).

We analysed the effect of geographic distance between sampling localities on the similarity of helminth component communities separately for each of the two hosts using multiple regressions on distance matrices (MRM; see Manly 1986; Legendre and Legendre 1998; Lichstein 2007 for

details). MRM is an extension of the partial Mantel analysis. It investigates relationships between a multivariate response distance matrix (here Bray-Curtis pairwise similarity in species composition of helminth infracommunities) and an explanatory distance matrix (pairwise geographic distances). Geographic distances between data points were measured in kilometres using the measure tool in ArcGIS 10.2.2 (ESRI) software. The significance of the model and regression coefficients was tested by permuting a response matrix while the explanatory matrices are held constant. The rows and corresponding columns in the response matrices are permuted simultaneously and the coefficient of determination of the model and regression coefficients are calculated for each permutation to generate a null distribution (Legendre and Legendre 1998; Lichstein 2007). All probabilities were based on 10000 permutations. Analyses were performed using the package "ecodist" (Goslee and Urban 2007) implemented in the R statistical environment (R Core Team 2016).

Results

Analysis of similarity of species composition of helminth infracommunities between the two host species resulted in $R = 0.08$ ($p < 0.001$). Significance of the test statistics for the analysis based on infracommunity composition suggests that the two hosts, in general, do not harbour identical helminth assemblages. Nevertheless, the low value of R indicates a broad overlap in species composition between helminth infracommunities of *R. pumilio* and *R. dilectus*. Furthermore, this overlap was broader than the overlap in helminth species composition among *R. pumilio* or *R. dilectus* from different localities (see below). Four helminth species contributed most to the dissimilarity between host species (cumulative contribution = 71.75%) due to either a difference in abundance between the two hosts or complete absence from one of the host species (e.g.,

Neoheligmonella sp. was recorded in *R. dilectus* but not *R. pumilio*) (Table 5.1). Between-host analysis of similarity based on component community composition resulted in R = 0.08 (p = 0.06), suggesting that species composition of helminth component communities between hosts was, on average, as similar as that among populations of conspecific hosts.

Table 5.1 Helminth species that contributed most to dissimilarity in species composition of helminth infracommunities between *Rhabdomys pumilio* and *R. dilectus*. AB – average abundance (after transformation and standardizing; see text for explanation).

Helminth species	AB in *R. pumilio*	AB in *R. dilectus*	Contribution to dissimilarity (%)
Heligmonina spira	33.27	6.54	24.45
Neoheligmonella capensis	8.02	28.15	23.78
Syphacia sp.	8.93	12.82	14.06
Neoheligmonella sp.	0.00	12.29	9.46

Global values of the test statistics R for among-localities and between-biomes similarity in helminth species composition of individual *R. pumilio* were 0.25 and 0.28, respectively, both being larger than any of 999 permuted values ($p < 0.0001$ for both). In other words, similarity in species composition of helminth assemblages was significantly lower among localities and between biomes than among host individuals within a locality. However, the degree of dissimilarity among localities was almost the same as that between biomes. In other words, communities differed more among localities and between biomes than within localities or biomes, respectively. Likewise, helminth infracommunities were more dissimilar among *R. dilectus* from different localities than among individuals from the same locality, but the value of the test statistics was much greater than that in *R. pumilio* ($R = 0.64$, $p < 0.001$). Helminth species that contributed most in pairwise dissimilarity between localities are presented in Table 5.2. The distribution of sampling localities in the ordination space constructed using non-parametric multidimensional scaling (MDS) is presented in Fig. 5.2. In *R. pumilio*, species composition of helminth infracommunities of populations inhabiting the Nama-Karoo and the Succulent Karoo differed sharply (along both the first and the second dimensions) from that of the populations in the Fynbos. In *R. dilectus*, species composition of helminth infracommunities differed the most between southern and northern populations.

Table 5.2 Helminth species that contributed most (maximum contribution > 20%) to the pairwise between-locality dissimilarity in species composition of infracommunities in *Rhabdomys pumilio* and *R. dilectus*. M - mean (across pairs of localities) percentage contribution to dissimilarity, MC – maximum contribution, N - number of locality pairs in which a given species contributed to dissimilarity.

Host species	Helminth species	M	MC	N
R. pumilio	*Aspiculuris shikoloueta*	24.83	33.91	17
	Neoheligmonella capensis	28.91	52.00	31
	Raillietina trapezoides	39.12	68.36	17
	Skrjabinotaenia sp. 4	17.35	20.16	5
	Skrjabinotaenia sp. 6	25.74	32.84	9
	Syphacia sp.	29.09	48.11	33
	Trichostrongylus probolurus	18.12	30.13	6
R. dilectus	*Trichostrongylus* sp.	16.95	23.45	3
	Syphacia sp.	18.62	29.84	22
	Heligmonina spira	18.65	37.78	17
	Meggittina baeri	20.77	21.08	9
	Raillietina trapezoides	24.83	25.20	9
	Neoheligmonella sp.	25.01	41.05	24
	Neoheligmonella capensis	32.04	40.81	30
	Abbreviata sp.	33.33	33.33	9

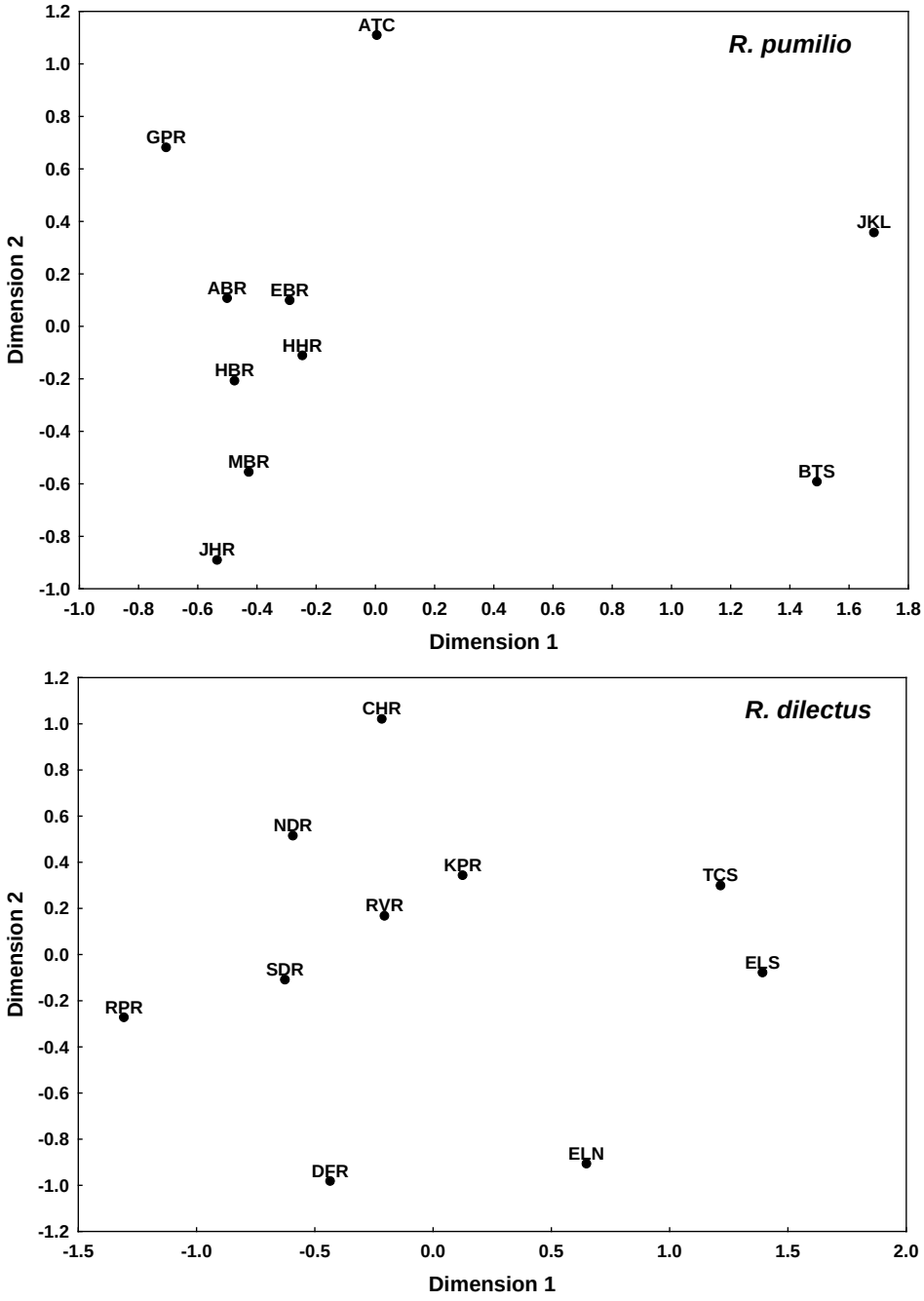

Fig. 5.2 Multidimensional scaling distribution of localities based on Bray-Curtis similarity in species composition of helminth infracommunities of *Rhabdomys pumilio* and *R. dilectus*. See Fig. 5.1 for abbreviations of the locality names.

MRM analysis demonstrated that similarity in helminth species composition among localities correlated significantly and negatively with geographic distance between localities ($r^2 = 0.64$, $F = 77.32$, $p = 0.0004$; coefficient = 0.001 for *R. pumilio* and $r^2 = 0.07$, $F = 3.27$, $p = 0.04$; coefficient = 0.0002 for *R. dilectus*). The farther two localities were from one another, the less similar their helminth assemblages were. Moreover, the rate of the decrease of similarity of helminth assemblages between a pair of localities with an increase in geographic distance between these localities was higher in *R. pumilio* than in *R. dilectus* (Fig. 5.3).

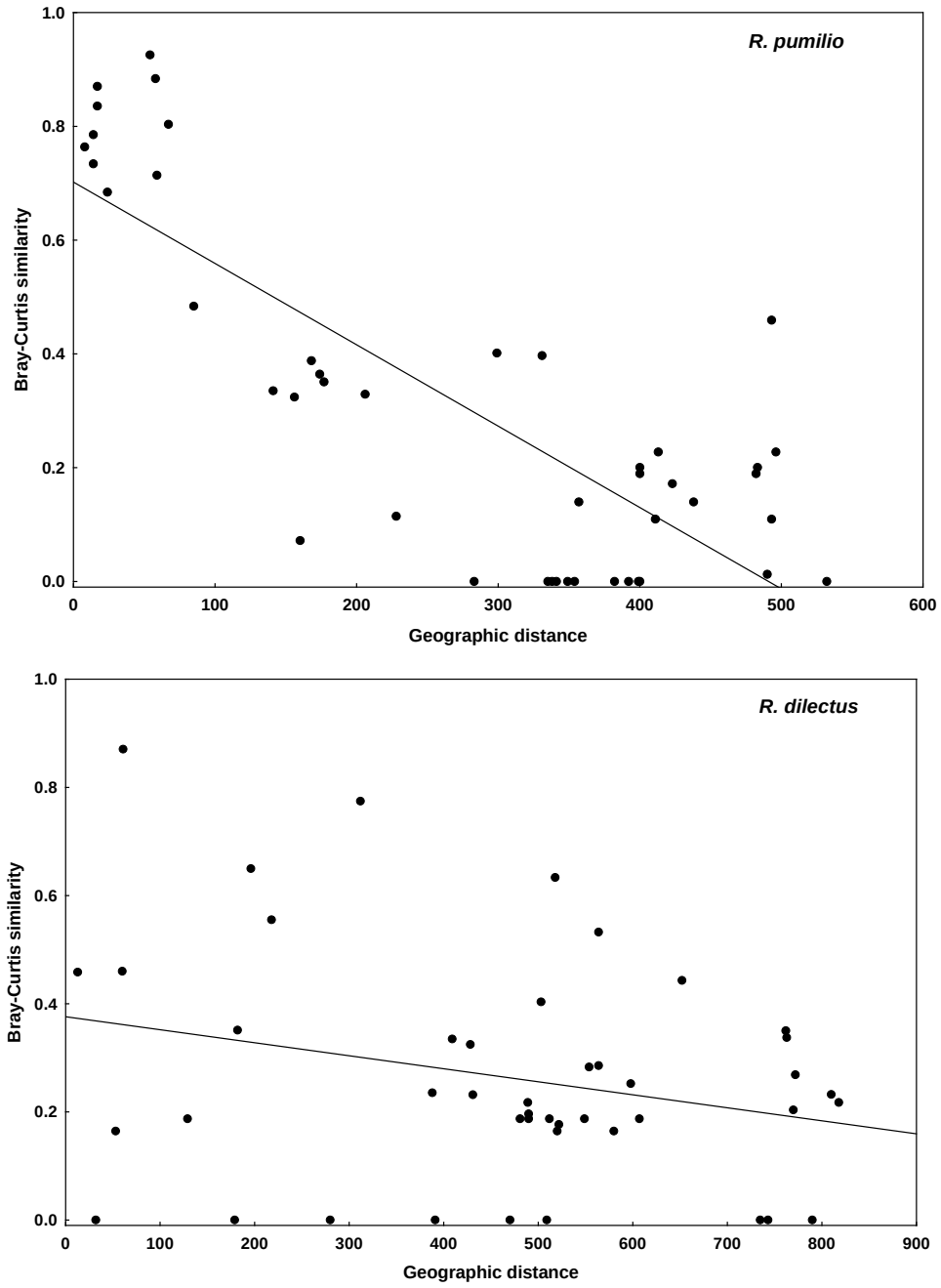

Fig. 5.3 Relationship between pairwise compositional dissimilarity of helminth component communities and pairwise geographic distance between sampling localities in *Rhabdomys pumilio* and *R. dilectus.*

Discussion

Our results suggest that factors associated with both host behaviour and environment drive spatial variation in helminth species composition in *R. pumilio* and *R. dilectus*. However, these factors differ in their relative importance.

Host behaviour

The sharpest contrast between the two host species found in this study was the much greater difference between (a) among-localities and (b) within-locality dissimilarity in helminth species composition in *R. dilectus* than in *R. pumilio*. This indicates that spatial variation in helminth composition was promoted mainly by the pattern of space use by the hosts rather than by their social behaviour. The reason behind this could be the greater extent of parasite exchange between host individuals within a given locality in *R. dilectus* than in *R. pumilio*. On the one hand, highly mobile *R. dilectus* that have large home ranges (Schradin and Pillay 2005a) likely encounter highly diverse vegetation types, microclimatic conditions, arthropod intermediate hosts and many other con- and heterospecific helminth hosts, being thus exposed to numerous and varied infective stages of helminth parasites. On the other hand, spatial distribution of *R. pumilio* within a locality is fragmented. Social groups within a locality are characterised by broad within-group overlap of home ranges and extremely weak or no home range overlap among groups (Schradin and Pillay 2005a). Consequently, although the rate of between-individual contacts within a group is presumably high, it is likely extremely low (if at all) among groups. In other words, individual *R. pumilio* are territorially conservative and move short distances only, thus, environmental conditions (including biotic interactions shaping species composition of their helminth assemblages) could be relatively stable for each given social group but vary among groups in the

same locality. Furthermore, although home ranges of individual *R. pumilio* belonging to the same social group broadly overlap, individuals from different groups exhibit a high level of aggression towards each other (Schradin and Pillay 2004), so that individual movements take place mainly within a group but not between groups. One of the results of this pattern could be a net high dissimilarity in helminth species composition of individual *R. pumilio* within a locality.

We found a significant decrease of similarity in species composition of helminth component communities with an increase of geographic distance between host populations. This is not especially surprising because distance decay of similarity has been documented for a variety of animal communities, including parasites (Poulin 2003; Bordes et al. 2011; Poulin et al. 2011b) and at a variety of spatial scales (Rohde 1992; Krasnov et al. 2005d; van der Mescht et al. 2016). The decrease of similarity in biological communities with distance can be explained by various mechanisms. One of these is a decrease in environmental similarity with increasing distance (Nekola and White 1999). Consequently, environmental similarity, including similarity in abiotic factors influencing egg and larval survival, infection opportunity and suitability for transmission results in similarity of helminth communities. Although distance decay of similarity in parasite communities is a rather general phenomenon, it is not universal. For example, Vinarski et al. (2007) established that the similarity in species composition of component communities of gamasid mites parasitic on small mammals across the Palearctic was generally unaffected by geographic distance between regions.

The rate of distance decay of similarity was higher in *R. pumilio* than in *R. dilectus*. In other words, the change in helminth species composition and/or their relative abundances per unit of distance

in *R. pumilio* was greater than that in *R. dilectus*. This difference can be explained by at least two reasons. First, it may be associated with between-host differences in vagility. The vagility of the host species is one of the key determinants of the range expansion or exchanges of parasites among host populations because of the limited dispersal abilities of the parasites themselves (Poulin 2003). Consequently, long distance movements of a host may lead to higher homogeneity of parasite communities. However, movements in both hosts are mainly limited to individual home ranges either within a social group (*R. pumilio*) or as solitary individuals (*R. dilectus*) (Schradin and Pillay 2003, 2004, 2005a, 2006; Schradin 2005). Despite that the dispersal ability of *R. dilectus* is approximately 10-fold that of *R. pumilio*, inter-locality dispersal is highly unlikely for both species. Any vagility-associated explanation of the difference in the rate of distance decay of similarity between two species would thus appear inadequate. Second, difference in the rate of distance decay of similarity between hosts might be linked to differences in the range of environmental variation across their geographic ranges. Environmental variation across the geographic range of a host may either promote or hamper dissimilarity of its parasite assemblages within this range. Habitat diversity across the geographic range of *R. pumilio* is more pronounced than that of *R. dilectus*. Indeed, the region inhabited by *R. pumilio* encompasses 23 vegetation zones, whereas that of *R. dilectus* covers 11 vegetation zones only (Mucina and Rutherford 2006). In addition, in the eastern, mesic parts of the country, suitable habitats for *R. dilectus* are more interconnected than those of *R. pumilio* in the western xeric regions, especially in the arid Karoo, so that populations of the latter species would be separated from each other by uninhabitable areas. Such habitat patchiness (Milton and Dean 1999) might accentuate differences in the helminth composition between different localities, as differences in helminth assemblages might evolve

faster through chance events of parasite loss on the one hand, and parasite acquisition from other rodents sharing the same habitat on the other hand (Sarà and Morand 2002; Bordes et al. 2015).

Environmental effects

Helminth infracommunities of each of the two hosts differed among localities to a somewhat greater extent than they differed between the two host species. This higher dissimilarity in helminth assemblages among localities within a host than between hosts suggests some effect of local environmental factors on helminth infracommunities. For example, vegetation structure may affect heteroxenous nematodes because they require suitable environments for egg survival and larval development (Hulbert and Boag 2001; Brouat et al. 2007). For example, poorer vegetation cover in xeric habitats of *R. pumilio* as compared to mesic habitats of *R. dilectus* may thus explain (at least, partly) the much higher abundance of *Neoheligmonella capensis* in the latter than in the former (Table 5.1). The same is likely the case for the presence of *Neoheligmonella* sp. in *R. dilectus* but not in *R. pumilio*.

Local environmental factors might affect helminth species composition of the same host among both different biomes and localities within the same biome. Moreover, these factors seem to be both climate- and vegetation-associated. Indeed, the degree of dissimilarity of helminth assemblages among localities was almost the same as that between biomes. Differential climate and vegetation could directly influence the free-living stages of parasitic helminths (Boomker et al. 2000; Turner and Getz 2010; Dybing et al. 2013). In South Africa, the mesic eastern region presents favourable conditions for free-living stages of parasitic nematodes, where they have a tendency to ascend vegetation in a film of moisture in order to become available to hosts (Anderson

2000). This would promote survival and transmission rates, whereas xeric conditions would cause desiccation of these stages. Arthropod diversity is influenced by environmental conditions, which may affect the presence of heteroxenous cestodes (e.g. *Meggittina baeri*) and some nematodes (*Abbreviata* sp.) that depend on arthropods as intermediate hosts for the completion of their life cycles (Anderson 2000; Georgiev et al. 2006). *Raillietina trapezoides* occurred only in the Karoo biomes, suggesting that habitat conditions elsewhere were unfavourable for its intermediate hosts. In contrast, *Syphacia* sp. is monoxenous and utilises a transmission strategy associated with behavioural patterns of its host (Taffs 1976; Anderson 2000). In addition to accidentally being ingested during foraging, it can also be transmitted via auto- and allo-grooming as well as retrofection, reducing its dependency on local environmental conditions. As a result, this helminth is present at all localities in both hosts and does not contribute much to the dissimilarity between host species as well as sampled localities.

However, we did not find strong support for our predictions regarding the direct effect of the level of moisture supply on spatial variation in helminth species composition. First, contrary to our expectation, the difference between within- and among-localities similarities was found to be greater in predominantly mesic *R. dilectus* than in predominantly xeric *R. pumilio*. Second, the differences between within-locality and, (a) among-localities or (b) Fynbos and Succulent Karoo biomes in the helminth composition of *R. pumilio* were essentially the same. These results, however, do not refute the effect of environment on helminth assemblages (see above). Indeed, MDS (Fig. 5.2.) demonstrated that helminth infracommunities in some localities differed substantially in species composition and/or their relative abundances from infracommunities in other localities. This was true for both host species. It seems that these differences might be driven

by factors other than the level of aridity; however, these factors remain undetermined and require further investigation.

Not identical, but similar helminth composition in both hosts

Despite the differences described above, *R. pumilio* and *R. dilectus* harboured similar, albeit not identical, helminth infracommunities. Moreover, similarities in species composition of component communities between and within hosts on average were the same. This suggests that both host species are equally susceptible to many helminth species. The most obvious reason behind this is close relatedness. The two species are relatively young and diverged ca 4.30 and 3.09 Ma, likely in response to vegetation and rainfall changes (du Toit et al. 2012). Morphological similarity of *R. pumilio* and *R. dilectus* can also be accompanied by similarity in physiological and immunological traits, although this has never been specifically studied.

Methodological aspects

The results of ANOSIM and tests for the distance decay of similarity seemed to be somewhat contradictory. For example, the ANOSIM results indicated higher among-locality heterogeneity in helminth communities of *R. dilectus*, whereas the rate of distance decay of similarity in species composition and/or relative abundance point to their higher homogeneity. One of the reasons behind this contradiction is that the two analyses captured different facets of spatial variation of helminth communities. Another reason can be that the results of the two analyses are not directly comparable. In fact, ANOSIM tests for the difference between within-locality and among-localities in the degree of similarity, whereas the test for distance decay of similarity compares communities among localities. Finally, ANOSIM dealt with infracommunities, whereas distance

decay of similarity was tested for component communities. The structure of these two hierarchical units is governed by different mechanisms (Poulin 2007a). In particular, this is because the former are ephemeral and short-lived, whereas the latter may persist for a long time.

In conclusion, our results demonstrated that differences in spatial utilization between the two hosts and, to a lesser extent, environmental conditions rather than host social behaviour determine patterns of spatial variation in species composition of their helminth assemblages.

General conclusions

The study explored the diversity and distribution of helminth parasites associated with small mammals in South Africa. This data then formed the basis for studies on the importance of host and environmental factors in shaping helminth infection and community structure in two closely related rodent host species, *R. pumilio* and *R. dilectus*.

Chapter 2 provides a faunistic, descriptive account of the helminth species that are associated with rodents in South Africa and provides base-line data on the distribution of helminths in space and time. Although shrews and sengis were included in the study the data was limited and thus no clear patterns were evident concerning these hosts. The study confirms the large diversity of helminth species associated with murid rodents and provides base-line data on possible host preferences. Several potentially new helminth species were recorded and the taxonomic placement of these species will need to be confirmed in future studies. Spatial distribution maps identified taxon-specific variation in geographic extent that is possibly driven by precipitation and vegetation cover. The importance of environmental condition in shaping helminth diversity and abundance is further supported in the fact that a larger number of helminth individuals and species were recorded in the months following the main rainfall period and a larger number of species were recorded in mesic (eastern summer rainfall region) compared to more xeric localities (western winter rainfall region). Based on the novelty of the data it is essential that future research should concentrate on an increased and equal sampling effort to attain a more diverse range of host species.

Chapter 3 investigates helminth parasitism in two closely related South African rodents, *R. pumilio* and *R. dilectus*, as to helminth abundance, prevalence, species richness and factors that influence these parameters. The study confirmed differences in the incidence of nematode infection between

these two spatially separate but closely related species, but the causality of sociality and environmental factors proved ambivalent. However, differences in nematode abundance and species richness between *R. pumilio* in the Fynbos and conspecifics from the Succulent/Nama Karoo biome suggested that the effect of environmental conditions on helminth infection is likely more important than the effect of sociality. Environmental factors such as precipitation and vegetation cover are considered as major contributors to differences in nematode and cestode infection between and within *Rhabdomys* species.

Chapter 4 continues the above comparison between the two closely related South African rodents by examining the possible effect of host sociality and spatial behaviour as drivers of non-random structuring of helminth communities. The general pattern of component helminth community co-occurrences was similar in the two hosts. Helminth infracommunities of *R. dilectus* and *R. pumilio* differed in the relative occurrence of positive and negative pairwise species co-occurrences. Nestedness-related patterns in helminth infracommunities were found in *R. pumilio* but not *R. dilectus*, whereas component communities of *R. dilectus* but not *R. pumilio* were significantly nested. Non-randomness in helminth assemblages was associated with the level of infection indicating that different facets of infection affected different structure patterns in the two hosts. The effects of host sociality and spatial behaviour on community structure of intestinal helminths could not be disentangled, but rather demonstrated a combination effect.

Chapter 5 elaborates on intra- and interspecific similarity in species composition of helminth communities in *R. pumilio* and *R. dilectus*. Factors associated with both host behaviour and the environment, although differing in relative importance, appear to drive spatial variation in

helminth species composition in *R. pumilio* and *R. dilectus*. *Rhabdomys pumilio* and *R. dilectus* harboured similar, albeit not identical, helminth infracommunities. However, a higher dissimilarity found in helminth infracommunity assemblages among localities within a host than between hosts suggests some effect of local environmental factors on helminth infracommunities. Similarities in species composition of component communities between and within hosts on average were the same which suggests that both host species are equally susceptible to many helminth species, close relatedness being the most obvious reason. In addition, a significant decrease of similarity in species composition of helminth component communities with an increase of geographic distance between host populations was confirmed, the rate of distance decay being higher in *R. pumilio* than in *R. dilectus*. It is evident that helminth parasite parameters and community structure are dependent on a complex interplay of host and parasite related factors, compounded by environmental influences.

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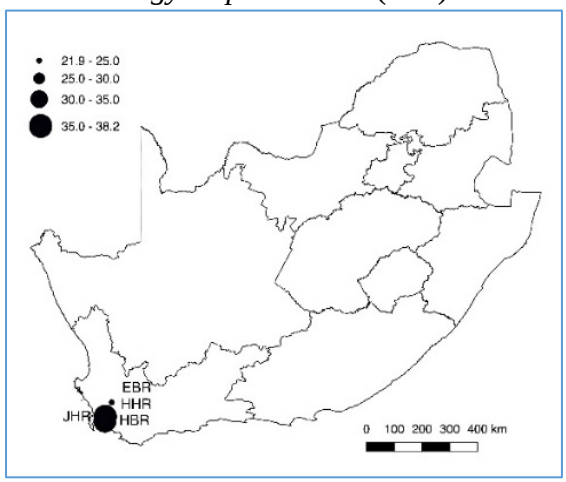
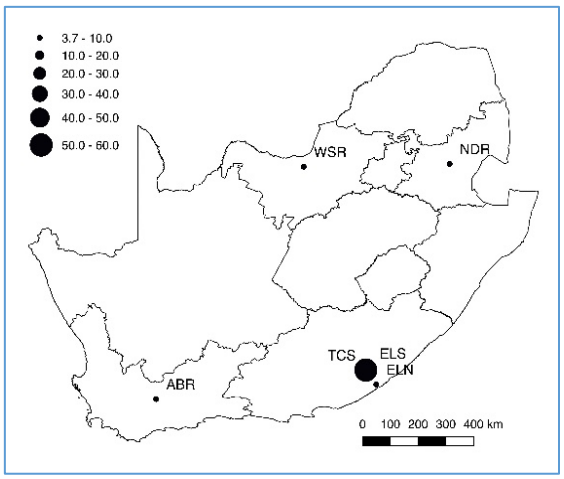
Appendix A

S. 1 Distribution and graphic indication of relative abundance of helminth species recorded in small mammals across South Africa. NM = nematodes with a direct life cycle; NH = nematodes with an indirect life cycle; CH = cestodes with an indirect life cycle.

Trichostrongylus probolurus (NM)



Trichostrongylus sp. (NM)



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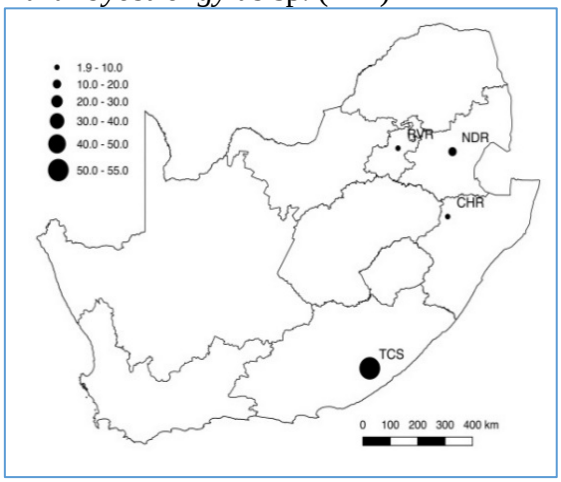
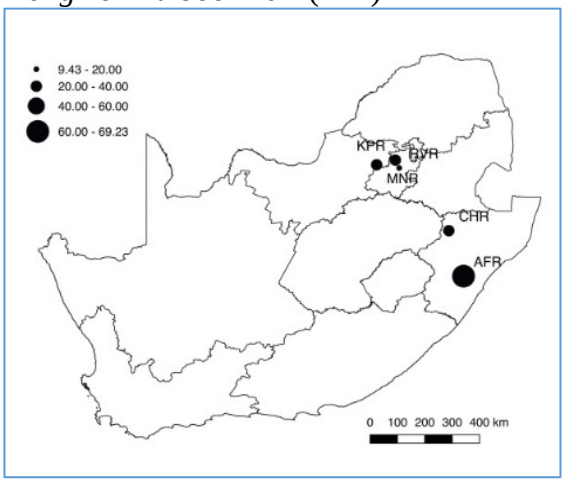
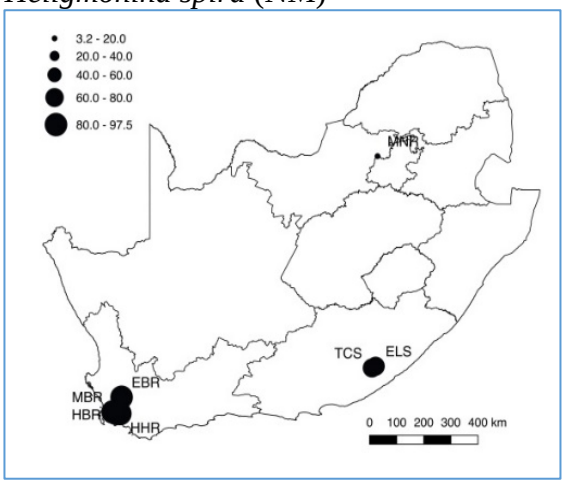


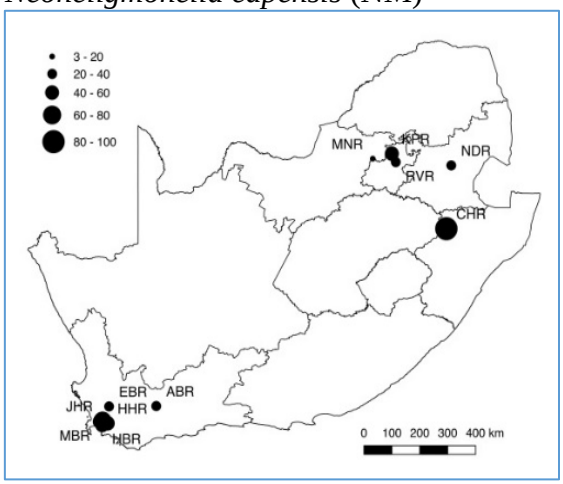
Heligmonina boomkeri (NM)



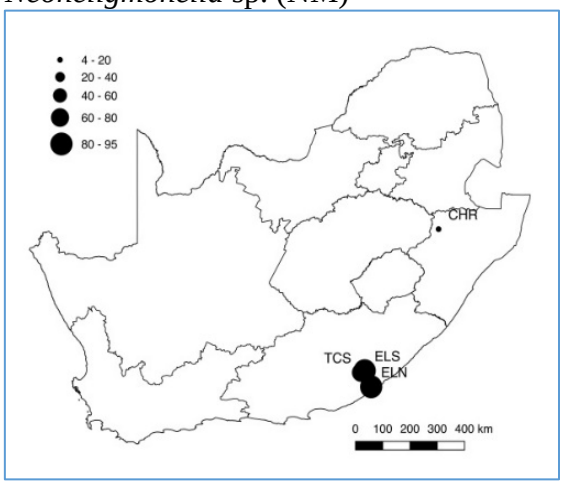
Heligmonina spira (NM)



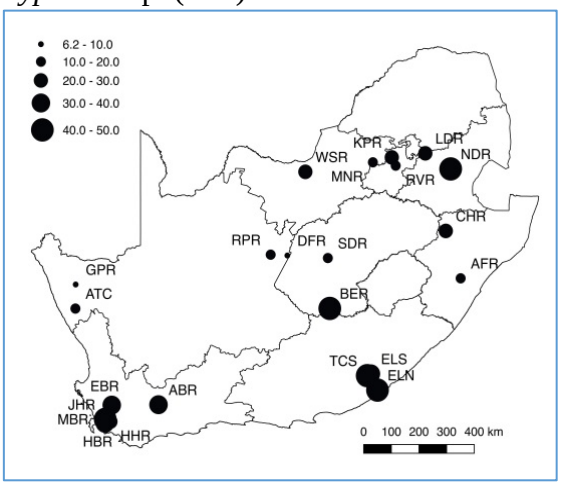
Neoheligmonella capensis (NM)



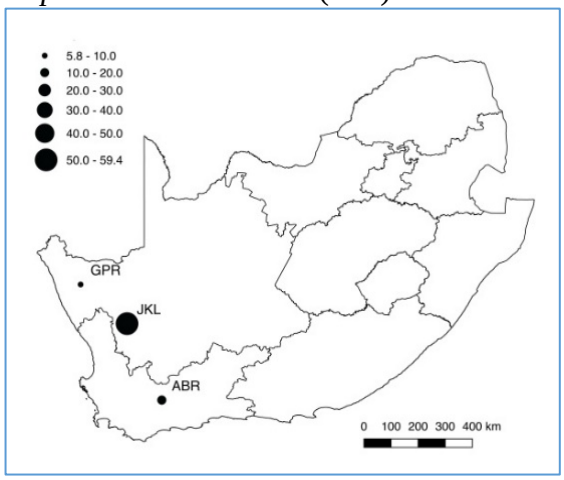
Neoheligmonella sp. (NM)



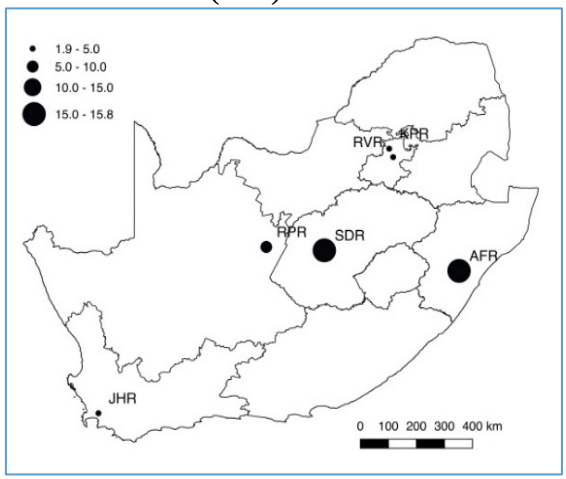
Syphacia sp. (NM)



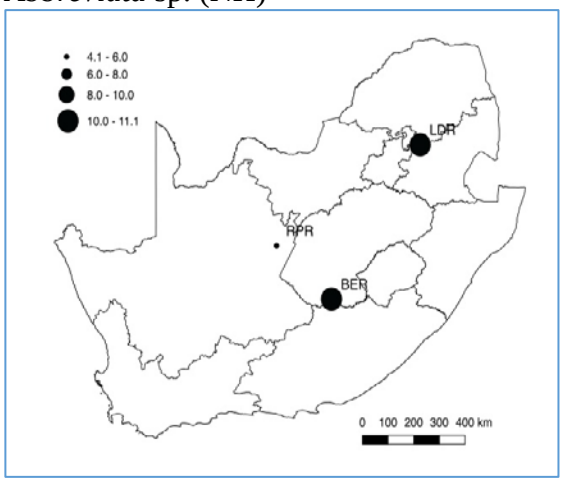
Aspiculuris shikoloueta (NM)



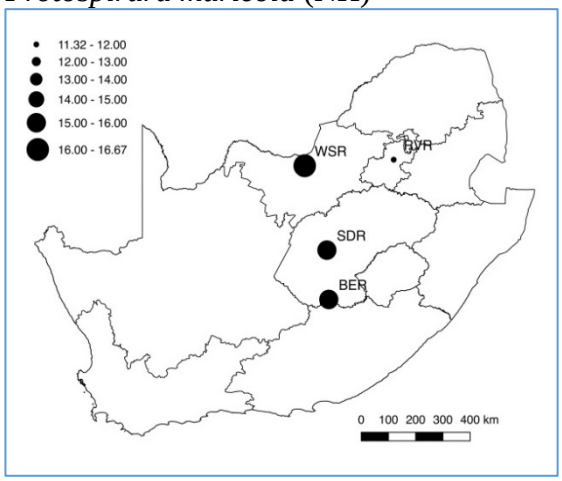
Trichuris muris (NM)



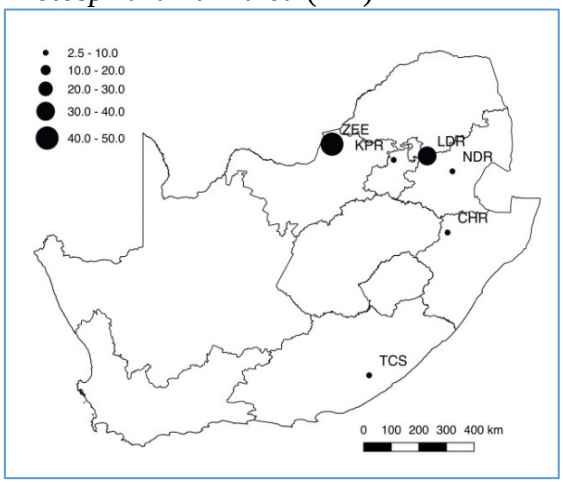
Abbreviata sp. (NH)



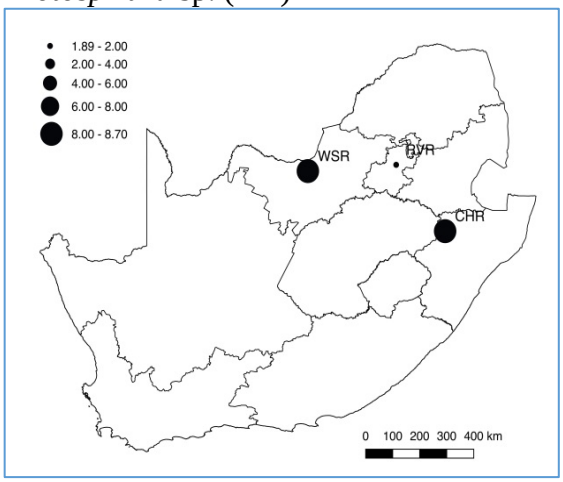
Protospirura muricola (NH)



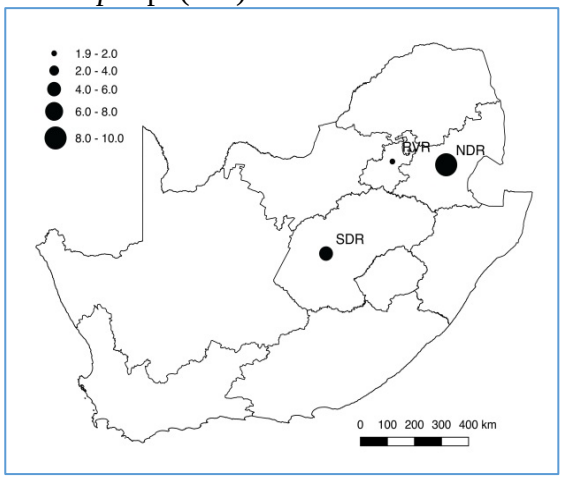
Protospirura numidica (NH)



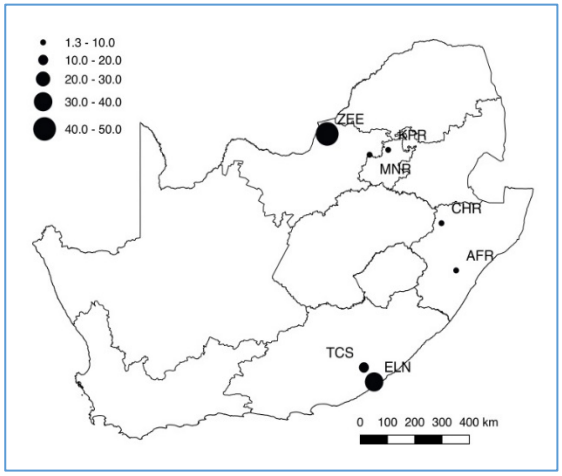
Protospirura sp. (NH)



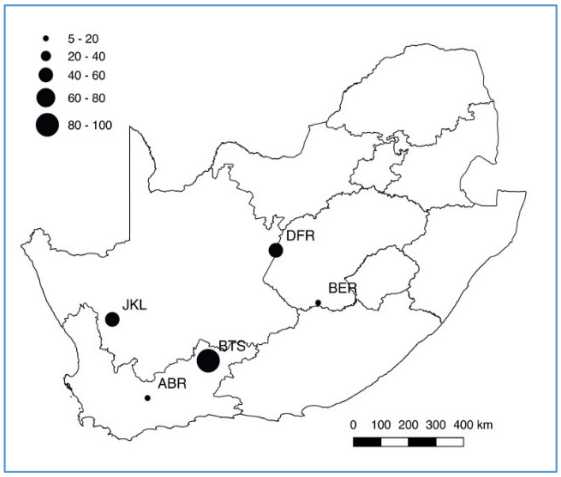
Ascarops sp. (NH)



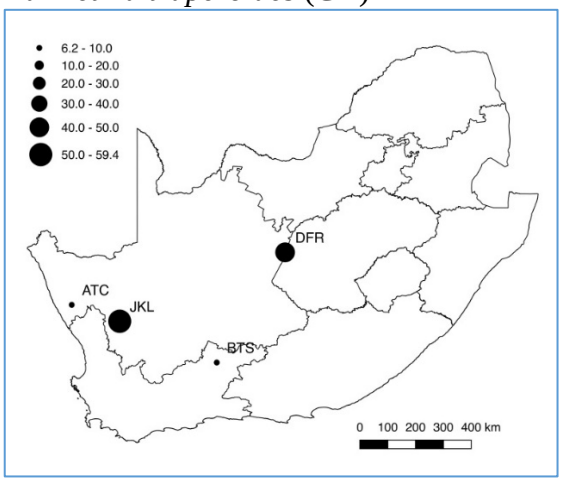
Inermicapsifer sp (CH)



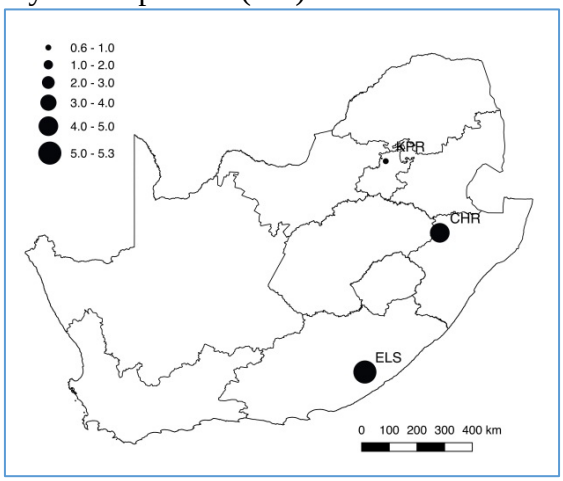
Meggittina baeri (CH)



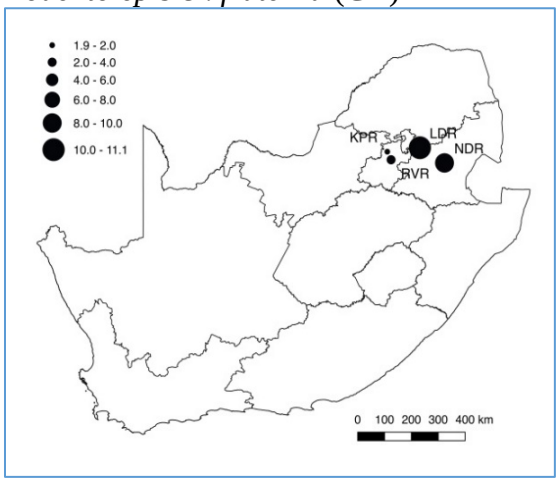
Raillietina trapezoides (CH)



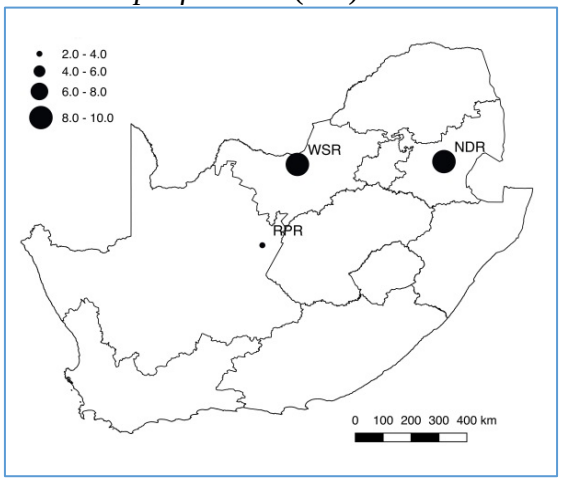
Hymenolepididae (CH)



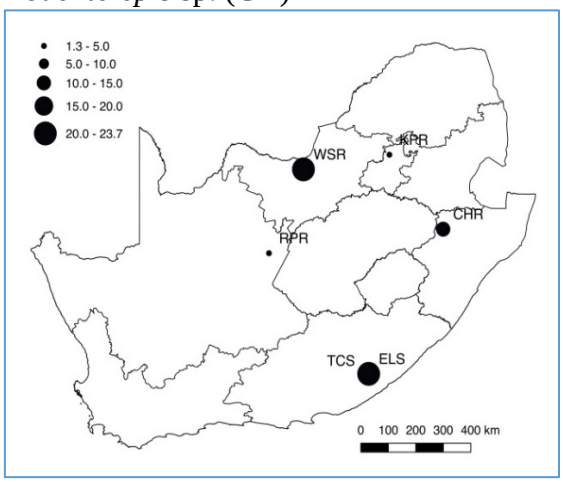
Rodentolepis cf. *fraterna* (CH)



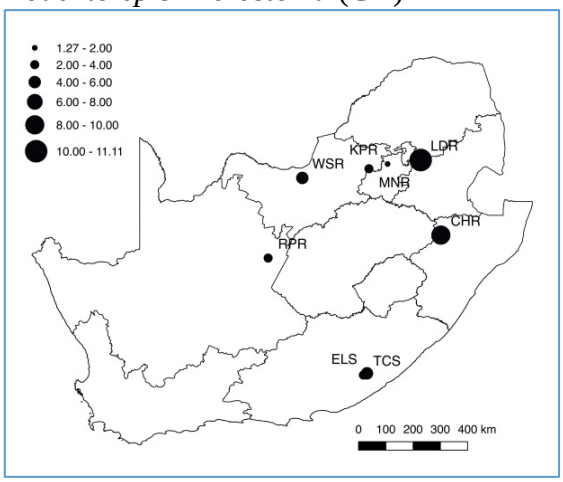
Rodentolepis fraterna (CH)



Rodentolepis sp. (CH)



Rodentolepis microstoma (CH)



Appendix B

S. 2 Localities where *Rhabdomys dilectus* and *R. pumilio* were sampled and examined for helminths. SS – number of hosts examined, TSR – number of helminth species, P – total prevalence of helminth infection.

Host/Locality	Abbreviation	Season	Latitude	Longitude	SS	TSR	P (%)
R. dilectus							
Nooitgedacht Dam Nature Reserve	NDR	summer	26.000	30.080	5	6	100.00
Kaalplaas Nature Reserve	KRP1	autumn	25.635	28.167	36	5	88.89
Kaalplaas Nature Reserve	KRP2	spring	25.635	28.167	6	4	50.00
Kaalplaas Nature Reserve	KRP3	summer	25.635	28.167	30	3	73.33
Kaalplaas Nature Reserve	KRP4	winter	25.635	28.167	25	3	72.00
Rietvlei Dam Nature Reserve	RVR	summer	25.897	28.294	24	5	79.17
Soetdoring	SDR	summer	28.823	26.089	19	5	42.11
Chelmsford Nature Reserve	CHR	winter	27.964	29.922	23	12	100.00
Dohne	ELS	summer	32.490	27.489	30	8	100.00
Croft	TCS	summer	32.547	27.368	25	10	100.00
Dronfield Nature Reserve	DFR	summer	28.742	24.772	32	4	62.50
Rooipoort Nature Reserve	RPR	summer	28.716	24.230	15	2	20.00
East London	ELN	winter	33.005	27.703	22	5	95.45
R. pumilio							
Klein Goegap	GRP1	autumn	29.660	17.893	31	1	9.68
Klein Goegap	GRP2	spring	29.660	17.893	28	2	32.14
Klein Goegap	GRP3	summer	29.660	17.893	30	1	6.67
Klein Goegap	GRP4	winter	29.660	17.893	31	2	6.45
Jakobskraal	JKL	summer	30.950	19.433	31	3	90.32
Agama Tented Camp	ATC	summer	30.423	17.884	27	4	51.85
Beaufort West	BTS	summer	32.271	22.565	15	3	100.00
Anysberg Nature Reserve	ABR	summer	33.467	20.584	27	6	70.37
Hottentots Holland Nature Reserve	HHR	summer	34.002	19.022	42	5	88.10
Jonkershoek Nature Reserve	JHR	summer	33.931	18.854	40	5	100.00
Muldersbosch	MBR1	autumn	33.949	18.766	34	5	97.06
Muldersbosch	MBR2	spring	33.949	18.766	25	5	100.00

Muldersbosch	MBR3	summer	33.949	18.766	28	6	96.43
Muldersbosch	MBR4	winter	33.949	18.766	31	5	54.84
Helderburg Nature Reserve	HBR	summer	34.055	18.844	34	4	79.41
Elandsberg Nature Reserve	EBR	summer	33.480	19.065	32	4	93.75

S. 3 Observed (O) and expected by chance (E; mean C-score of 5000 simulated matrices) values of *C*-score for presence/absence matrices of helminth communities. SES – standardized effect size, *P*-value is related to the difference between observed and expected values of the *C*-score (O < E if SES is negative or O > E if SES is positive). Null matrices were constructed using the equiprobable-equiprobable algorithm. See Appendix B, Table S.2 for abbreviations of the locality names.

Host	Locality	*C*-score		SES	*P*
		O	E		
R. dilectus	CHR	3.48	11.8	-10.32	<0.001
	DFR	6.83	43.65	-5.37	<0.001
	ELN	7.6	25.65	-4.73	<0.001
	ELS	5.64	48.5	-11.43	<0.001
	TSC	2.95	37.66	-12.9	<0.001
	KRP1	28	45.24	-1.71	<0.001
	KRP2	15.3	59.42	-6.46	<0.001
	KRP3	5.66	27.66	-3.22	0.04
	KRP4	1.33	0.88	0.78	0.82
	RVR	10.2	18.6	-3	0.004
	NDR	0.8	1.29	-1.07	0.17
	SDR	3	2.87	0.21	0.56
R. pumilio	ABR	12.66	16.76	-2.09	0.02
	ATC	13.17	12.17	0.45	0.66
	BTS	0	13.3	-3.06	<0.001
	EBR	5.16	61.44	-6.05	<0.001
	HBR	19	70.31	-4.91	<0.001
	HHR	10.83	106.7	-6.82	<0.001
	JHR	32.3	97.83	-6.45	<0.001
	JKL	54.33	54.53	-0.02	0.50
	MBR1	29.5	67.36	-4.89	<0.001
	MBR2	16.1	36.96	-4.14	<0.001
	MBR3	12.87	43.14	-6.49	<0.001
	MBR4	7.90	19.03	-7.21	<0.001

S. 4 Results of linear mixed-effects models of the effect of mean helminth intensity (I), species richness (SR), prevalence (P) and matrix size (MS) on absolute values of SES for helminth species co-occurrence in *Rhabdomys dilectus* (RD) and *R. pumilio* (RP). df – degrees of freedom, LogLik – log-likelihood test, AICc – Akaike Information Criterion (AIC) corrected for sample size, AICw – AIC weight.

Species	Model no.	Coefficients					df	LogLik	AICc	ΔAIC	AIC w
		Intercept	I	SR	P	MS					
RD	1	0.29		6.02			5	-32.27	84.50	0.00	0.24
	2	-0.90				0.09	4	-35.61	84.90	0.40	0.20
	3	-4.43			5.04	0.09	5	-32.51	85.00	0.48	0.19
	4	-0.88		5.74			4	-35.76	85.20	0.70	0.17
	5	-1.75			16.23		4	-36.35	86.40	1.89	0.09
	6	-3.07		1.89		0.08	5	-33.52	87.00	2.50	0.07
	7	-3.53		1.77	0.84	0.08	6	-30.50	89.80	5.27	0.02
	8	11.27					3	-40.64	90.30	5.74	0.01
	9	7.17	0.07		-0.88		5	-35.70	91.40	6.86	0.01
	10	6.53	0.07				4	-38.97	91.60	7.11	0.01
	11	-0.14	0.03			0.07	5	-36.67	93.30	8.81	0.00
	12	4.62	0.06	1.26			5	-36.84	93.70	9.13	0.00
	13	6.25	0.06	1.60	-2.98		6	-33.41	95.60	11.09	0.00
	14	0.40	0.03		-0.74	0.07	6	-33.90	96.60	12.06	0.00
	15	0.63	0.04	-0.58		0.07	6	-35.05	98.90	14.36	0.00
	16	0.61	0.04	-0.58	0.03	0.07	7	-32.13	106.30	21.73	0.00
RP	1	-11.88			25.21		4	-32.48	78.70	0.00	0.52
	2	-14.55		3.45	20.11		5	-29.79	79.60	0.89	0.33
	3	-5.15		7.24			4	-34.51	82.70	4.04	0.07
	4	-24.70			25.32	0.09	5	-31.72	83.40	4.75	0.05
	5	9.57					3	-38.35	85.70	7.02	0.02
	6	-25.33		-4.85	32.54	0.12	6	-28.70	86.20	7.51	0.01
	7	-3.09				0.09	4	-38.63	91.00	12.29	0.00
	8	-7.76		4.10		0.07	5	-35.79	91.60	12.89	0.00
	9	-9.57	0.00		21.13		5	-36.94	93.90	15.20	0.00
	10	-11.78	0.00	1.99	19.36		6	-34.29	97.40	18.70	0.00

	11	7.36	0.01				4	-41.91	97.50	18.85	0.00
	12	-1.86	0.00	5.06			5	-38.86	97.70	19.05	0.00
	13	-24.86	0.00		25.47	0.09	6	-36.72	102.20	23.57	0.00
	14	-1.96	0.00			0.07	5	-42.90	105.80	27.12	0.00
	15	-23.38	0.00	-5.69	31.89	0.12	7	-33.54	109.10	30.40	0.00
	16	-4.58	0.00	2.06		0.06	6	-40.17	109.10	30.46	0.00

S. 5 Results of linear mixed-effects models of the effect of mean helminth intensity (I), species richness (SR), prevalence (P) and host abundance (HA) on absolute values of SES for helminth species co-occurrence in *Rhabdomys dilectus* (RD) and *R. pumilio* (RP). df – degrees of freedom, LogLik – log-likelihood test, AICc – Akaike Information Criterion (AIC) corrected for sample size, AICw – AIC weight.

Species	Model no.	Coefficients					df	LogLik	AICc	ΔAIC	AIC w
		Intercept	I	SR	P	HA					
RD	1	-0.64					4	-5.42	28.80	0.00	0.50
	2	1.03			2.06		3	-9.10	29.00	0.17	0.46
	3	0.22	0.49				4	-8.39	34.80	5.94	0.03
	4	0.56		0.20			4	-9.29	36.60	7.73	0.01
	5	-0.43	-0.43		2.67		5	-4.84	39.70	10.83	0.00
	6	1.63				-0.03	4	-10.98	40.00	11.11	0.00
	7	-0.64		-0.14	2.47		5	-5.86	41.70	12.87	0.00
	8	-0.04			2.05	-0.03	5	-6.53	43.10	14.23	0.00
	9	0.27	0.38	0.06			5	-8.39	46.80	17.95	0.00
	10	0.68	0.65			-0.04	5	-9.80	49.60	20.75	0.00
	11	1.11		0.26		-0.04	5	-10.79	51.60	22.73	0.00
	12	-0.47	-0.36	-0.04	2.69		6	-5.13	64.30	35.41	0.00
	13	0.01	-0.18		2.31	-0.03	6	-6.41	66.80	37.98	0.00
	14	-0.06		-0.05	2.20	-0.03	6	-7.36	68.70	39.87	0.00
	15	0.74	0.49	0.09		-0.04	6	-9.92	73.80	44.99	0.00
	16	0.01	-0.17	0.00	2.31	-0.03	7	-6.89	139.80	110.94	0.00
RP	1	2.95					3	-20.28	50.60	0.00	0.52
	2	3.61			-0.80		4	-18.01	52.00	1.45	0.25
	3	3.51		-0.27			4	-18.97	53.90	3.38	0.10
	4	3.79	-0.37				4	-19.39	54.80	4.21	0.06
	5	-3.10				0.19	4	-20.16	56.30	5.74	0.03
	6	3.64		-0.07	-0.65		5	-16.19	57.40	6.81	0.02
	7	3.33	-0.58		1.15		5	-16.58	58.20	7.59	0.01
	8	2.37	-1.36	1.76			5	-17.02	59.00	8.48	0.01
	9	-1.64			-2.83	0.22	5	-17.65	60.30	9.74	0.00

	10	-1.93		-0.76		0.20	5	-18.79	62.60	12.01	0.00
	11	-2.18	-0.75			0.21	5	-19.02	63.00	12.47	0.00
	12	2.21	-1.43	1.69	0.55		6	-14.14	68.30	17.71	0.00
	13	-1.71		0.13	-3.10	0.22	6	-15.96	71.90	21.36	0.00
	14	-1.93	-0.60		-0.84	0.22	6	-16.33	72.70	22.09	0.00
	15	-3.94	-1.92	2.06		0.22	6	-16.71	73.40	22.85	0.00
	16	-3.63	-1.74	2.28	-1.72	0.22	7	-13.91	97.80	47.26	0.00